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14. ABSTRACT

The U.S. Air Force Medical Service presented the sixth annual Air Force Medical Research Symposium coordinated by the Air Force Medical Support Agency's Research and Development Division (AFMSA/SGRS). The symposium was held 2-4 August 2011 at the Gaylord National Hotel & Convention Center, National Harbor, MD. The symposium featured two half-days of plenary sessions, one and a half days of scientific presentations, and a poster session. It was organized into five tracks to include: Operational Medicine (In-Garrison Care), Enroute Care and Expeditionary Medicine, Force Health Protection, Traumatic Brain Injury (TBI) and Psychological Health, and Healthcare Informatics. These proceedings are organized into six volumes to include one that provides a general overview and all presentation and poster abstracts; the other five each address a specific track. Volume 3 contains abstracts and presentation slides for the Force Health Protection Track.

15. SUBJECT TERMS

US Air Force, Medical Service, Medical Research, Force Health Protection

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Proceedings of the 2011 AFMS Medical Research Symposium

Volume 3. Force Health Protection Track Abstracts and Presentations

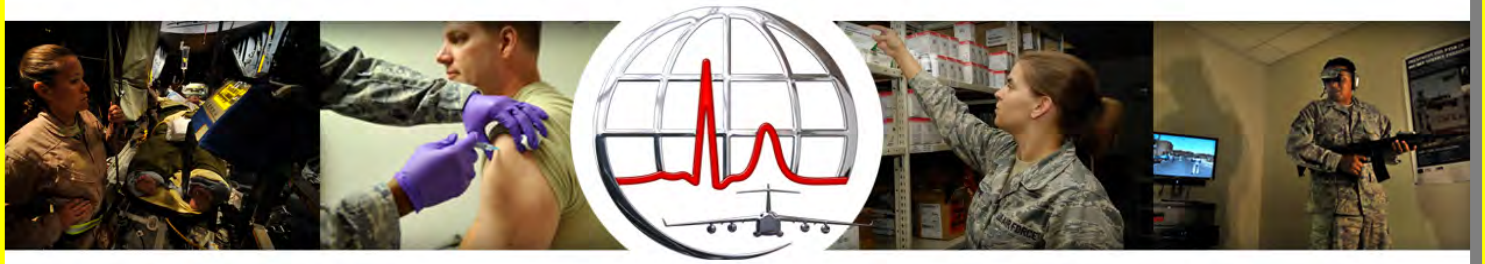
Volume 3 of 6

AIR FORCE MEDICAL SERVICE

2011 AFMS Medical Research Symposium

2-4 August 2011

GAYLORD NATIONAL
201 Waterfront Street
National Harbor, MD 20745
(1-877-677-9352)



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Proceedings of the 2011 AFMS Medical Research Symposium
Volume 3. Force Health Protection Track Abstracts and Presentations

Edited by: Dr. Welford C. Roberts

Held
2-4 August 2011
at the
Gaylord National Resort Hotel and Convention Center
201 Waterfront Street
National Harbor, MD 20745

Table of Contents

Proceedings of the 2011 AFMS Medical Research Symposium

Introduction

The U.S. Air Force Medical Service presented the sixth annual Air Force Medical Research Symposium coordinated by the Air Force Medical Support Agency's Research and Development Division (AFMSA/SGRS). The symposium was held on 2-4 August 2011 in the Washington DC area at the Gaylord National Resort Hotel and Convention Center in National Harbor, MD. The symposium featured two half-days of plenary sessions, one and a half days of scientific presentations, and a poster session.

The symposium was organized into several tracks to include Enroute Care, Force Health Protection, Healthcare Informatics, Operational Medicine (In-Garrison Care), and Psychological Health/Traumatic Brain Injury, as follows:

- The Enroute Care Track addressed science and technology targeted at the continuum of care during transport from point of injury to definitive care including, but not limited to: Casevac, Medivac; Aeromedical Evacuation; Critical Care Air Transport; and Patient Staging. Further areas addressed included: patient stabilization; patient preparation for movement; impact of in-transit environment on patient and AE crew physiology; human factors concerns for AE crew or patient population; AE/medical personnel training; infectious disease/control; burn management; pain management; resuscitation; lifesaving interventions; and nutrition research in the enroute care environment.
- The Force Health Protection Track focused on prevention of injury and illness and the early recognition or detection of emerging threats for in-garrison or deployed operations. Topics of interest include research in bio-surveillance, infectious disease, emerging threats (pandemic response), protective countermeasures, disaster response/consequence management, toxicology/health risks (e.g., particulates nanomaterials, radiation, etc.), monitoring disease trends, other areas of preventive medicine, public and environmental health relevant to the military workforce.
- The Healthcare Informatics Track focused on the use of innovative information management & technology solutions that enhance healthcare delivery at any point of the full spectrum of patient care to include medical simulation and training.
- The Operational Medicine (In-Garrison Care) Track focused on care delivered in the outpatient or inpatient in-garrison setting and on enhancing the performance of airman in challenging operational and expeditionary environments.
- The Psychological Health/Traumatic Brain Injury Track addressed topics pertaining to screening, diagnosis, and treatment of TBI and/or Psychological Health in the military community. Specific focus areas within Psychological Health included depression, substance use disorders, family functioning, and suicide prevention. Topics of special interest included field-deployable diagnostic tests for mild TBI (concussion), blast modeling, large epidemiologic studies of Psychological Health and TBI, and strategies for translating research into practice.

These proceedings are organized into five volumes, as follows:

- Volume 1. This volume is a general overview of the entire 2011 Air Force Medical Research Symposium and includes abstracts of all the oral presentations and posters. First presented is the symposium's opening plenary session, followed by the abstracts from the four technical tracks, and then the closing plenary session. The abstracts associated with the poster session are in the last section of these proceedings. The agenda for the overall symposium is in Appendix A, attendees are listed in Appendix B, and continuing education information is in Appendix C of this volume. Appendices D-J are copies of presentation slides from the plenary sessions.
- Volume 2. This volume contains abstracts and presentation slides for the Enroute Care Track.
- Volume 3. This volume contains abstracts and presentation slides for the Force Health Protection Track.
- Volume 4. This volume contains abstracts and presentation slides for the Healthcare Informatics Track.
- Volume 5. This volume contains abstracts and presentation slides for the Operational Medicine (In-Garrison Care) Track.
- Volume 6. This volume contains abstracts and presentation slides for the Psychological Health/Traumatic Brain Injury Track

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Air Emissions Characterization and Geospatial Exposure Modeling from Open Burning of Representative Military Deployed Waste

AF Institute of Technology

Lt Col Dirk Yamamoto

Open burning of US military waste while deployed has attracted considerable attention over recent years due to reported health problems among returning military members. In conjunction with the rest of DoD, the US Air Force has conducted considerable sampling and risk assessment at deployed sites. At the Air Force Institute of Technology (Wright-Patterson AFB, OH), recent research has focused on building a retrospective plume dispersion modeling tool for particulate matter exposures, to better characterize the risk profile for deployed members. This approach may provide more realistic exposure estimates, versus assigning a single exposure value for an entire population. Ongoing research, sponsored by AF Surgeon General and performed in conjunction with the US Environmental Protection Agency, will first determine emission factors and likely concentrations of key contaminants by performing small-scale laboratory burns, with subsequent large-scale outdoor burns to evaluate the effectiveness of air curtain burners as an alternative to open/surface burns. A primary objective of the research is to address the question on whether segregation of plastics makes a significant difference in emissions from open- and air curtain burning. A secondary objective is to further develop the software plume dispersion modeling tool to better predict downwind risk to personnel near burn sites. This presentation provides a status update of the ongoing research at the Air Force Institute of Technology.

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Burn Pit Waste: Air Emissions Characterization

AF Medical Research Symposium, 2 Aug 2011

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U.S. AIR FORCE

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Disclaimer

"The views expressed in this presentation are those of the author and do not reflect the official policy or position of the United States Air Force, Department of Defense, or the U.S. Government."

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Outline

- Background
- AFIT Dispersion Modeling
- Small-Scale Emissions Tests
- Large-Scale Emission Tests

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Background

- Open pit burning→ expediency
  - Simple, relatively inexpensive
  - Less force protection: no off-the-base convoy
  - Vector-borne disease control
  - Aesthetics/odors
- Concerns
  - Emissions → potential health risk

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Background

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- 4-10 pounds of waste per soldier, per day
- Alternatives include:
  - Reduction
  - Reuse
  - Recycling
  - Incinerators
  - Burn boxes

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Pollutants of Concern

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- Particulate Matter: PM_{10}, $PM_{2.5}$
  - Ambient: typically above levels seen stateside
    - Naturally-occurring, man-made
- Metals:
  - In native soil
  - From burning
- Dioxins/Furans:
  - Low-temperature burning, especially from plastics

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Pollutants of Concern (con't.)

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- Polycyclic Aromatic Hydrocarbons (PAHs):
  - Incomplete, low-temperature combustion of organics
- Volatile Organic Compounds (VOCs)
  - Combustion of organics
  - Fueling operations, etc.

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- "Newly Reported Respiratory Symptoms and Conditions Among Military Personnel Deployed to Iraq and Afghanistan: A Prospective Population-based Study" (Smith, 2009).
  - "Deployers had a higher rate of newly reported respiratory symptoms than nondeployers (14% vs. 10%)"
  - "Deployment length was linearly associated with increased symptom reporting in Army personnel"
  - "Specific exposures, rather than deployment in general," are strongly suggested "determinants of post-deployment respiratory illness."

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Background
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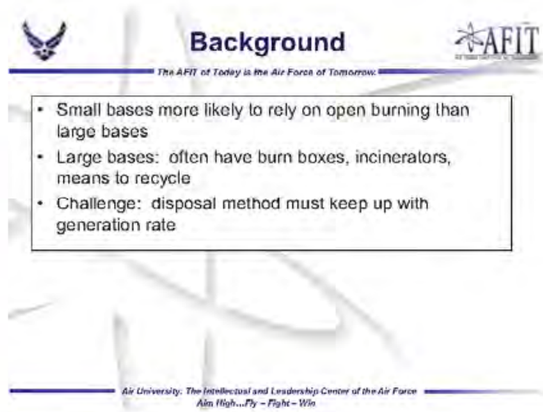
AFIT
• Balad burn pits have closed…
• Attention has shifted to Afghanistan, where open burning remains a common practice
Number of Burn Pits in Iraq & Afghanistan
Iraq
Afghanistan
67
Nov 09
Apr 10
Aug 10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• Small bases more likely to rely on open burning than large bases
• Large bases: often have burn boxes, incinerators, means to recycle
• Challenge: disposal method must keep up with generation rate
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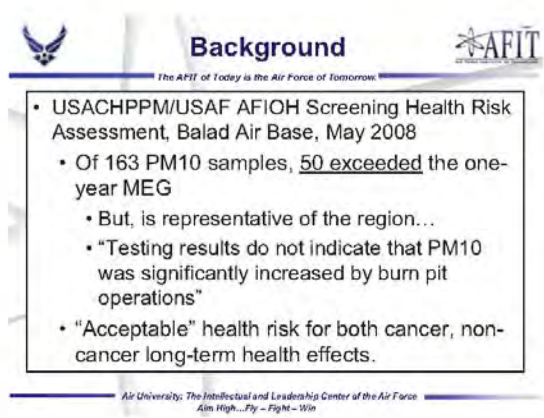
Background
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• USACHPPM/USAF AFIOH Screening Health Risk Assessment, Balad Air Base, May 2008
• Of 163 PM10 samples, 50 exceeded the one-year MEG
• But, is representative of the region…
• "Testing results do not indicate that PM10 was significantly increased by burn pit operations"
• "Acceptable" health risk for both cancer, non-cancer long-term health effects.
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Background[1]

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- October 2009: NDAA FY10, Section 317
  - Requires DoD to prescribe regulations prohibiting disposal of 'covered wastes' in open-air burn pits, except when SecDef determines no alternative
  - 'Covered waste': includes haz waste, regulated medical waste, tires, treated wood, batteries, PCBs, petroleum, oils, lubricants, asbestos, mercury, etc.
- March 2010: DoD issued DTM 09-032 to echo Section 317
  - Commanders given discretion to conduct open air burning when deemed necessary…

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Disposal Methods

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- Solid waste incinerators
  - Dual combustion chambers, emissions stack
  - Enclosed combustion chambers→more complete burn
- Burn boxes: air curtain or Munson burners
  - Designed for wood waste, not necessarily food/plastic
- Burn pits/surface burns
- Landfills
  - Lined
  - Unlined

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Incinerator

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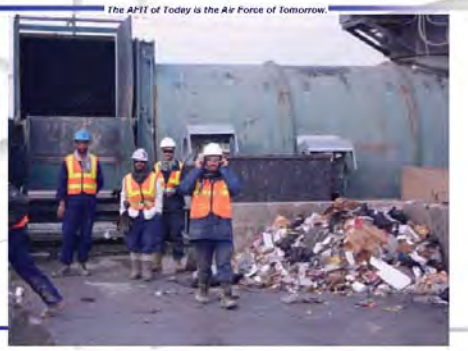

Burn Box

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Surface Burn
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Recycling
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GAO Report, Oct 2010
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Key findings:
• DoD should improve adherence to guidance
• e.g., plastics were being burned, not resourced/trained to sort trash
• Slow to implement incinerators, other alternatives
• Contracts didn't reflect current guidance
• Long-term health implications cannot be ruled out
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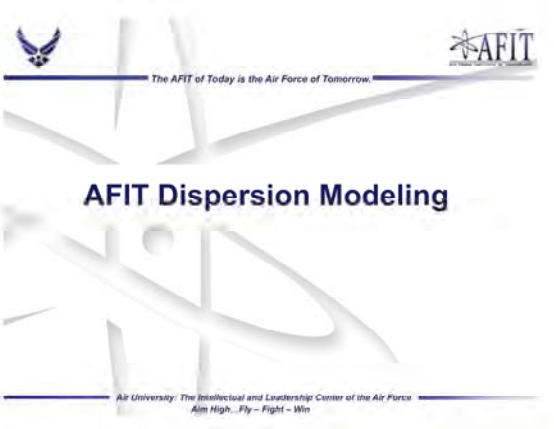
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AFIT Dispersion Modeling
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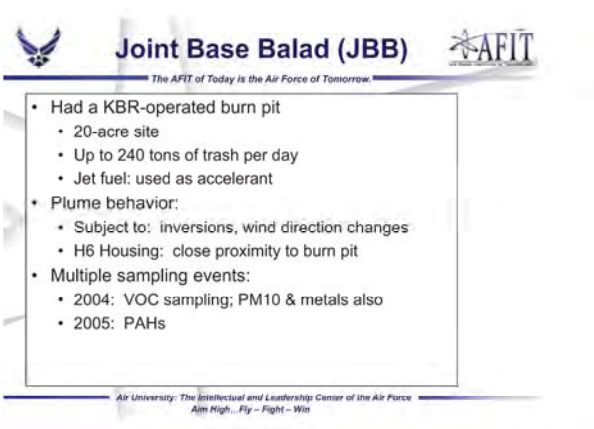
Joint Base Balad (JBB)
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• Had a KBR-operated burn pit
• 20-acre site
• Up to 240 tons of trash per day
• Jet fuel: used as accelerant
• Plume behavior:
• Subject to: inversions, wind direction changes
• H6 Housing: close proximity to burn pit
• Multiple sampling events:
• 2004: VOC sampling; PM10 & metals also
• 2005: PAHs
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JBB Burn Pit
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Google
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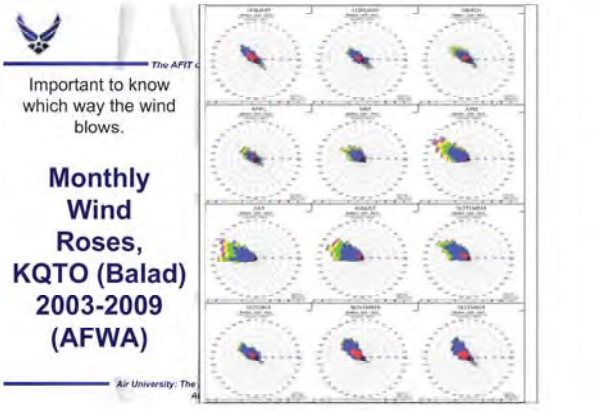
Important to know which way the wind blows.
Monthly Wind Roses, KQTO (Balad) 2003-2009 (AFWA)

JBB- Sampling Locations

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Data Example

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- Ideally: all analytes, all sites, all days
  - With meteorological data known
- Reality: select analytes, certain sites, certain days

Daily (PM10 levels in ug/m3)

SITE ID	22-Apr-03	24-Apr-03	28-Apr-03	29-Apr-03	31-Mar-04	1-Apr-04	2-Apr-04
A			71.81	73.89			
B						246.42	264.46
C							
D							
E							
F							
G	73.16	248.25					
I					83.01	131.49	329.77
J							
K							

AFIT Burn Pit Research

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- Student research by MSgt John Rinker
  - Retrospective geospatial modeling of burn pit emissions at Joint Base Balad (JBB): PM10
  - Create geospatially-enabled plume (dispersion) model to provide spatiotemporal exposure bands
    - Exposure contours
    - e.g., not everyone at JBB had the same exposure (not a single exposure group)

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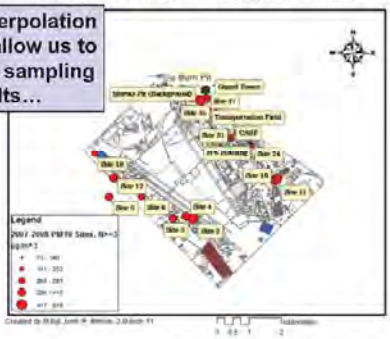
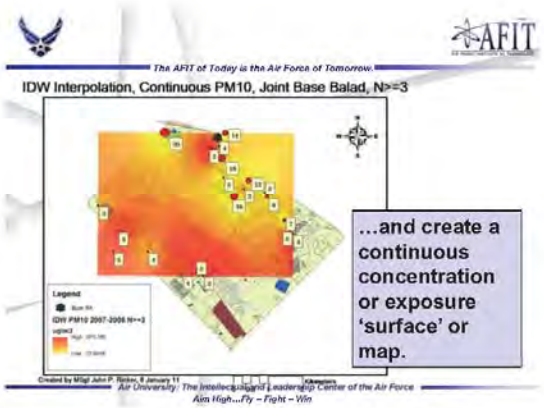
PM10 Geometric Mean at Balad Sampling Sites, 2007-2008

Spatial interpolation methods allow us to take point sampling results…

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IDW Interpolation, Continuous PM10, Joint Base Balad, N>=3
...and create a continuous concentration or exposure 'surface' or map.
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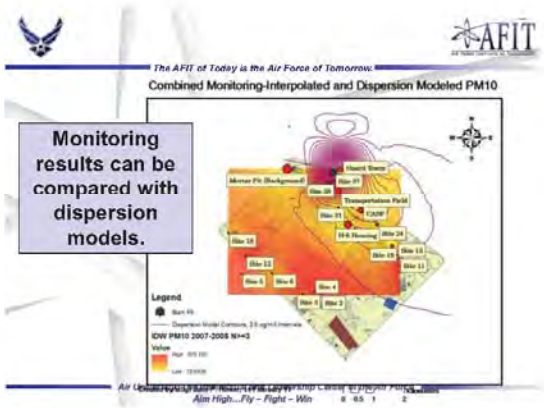
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Combined Monitoring-Interpolated and Dispersion Modeled PM10
Monitoring results can be compared with dispersion models.
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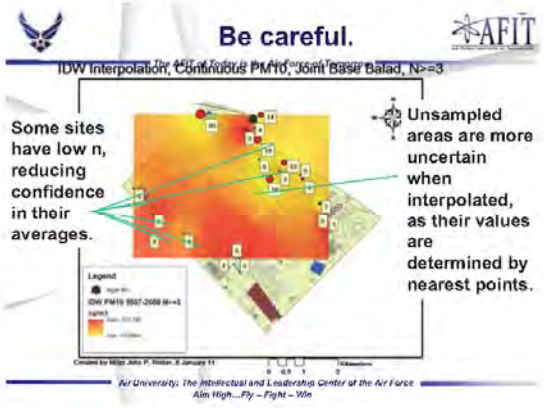
Be careful.
IDW Interpolation, Continuous PM10, Joint Base Balad, N>=3
Some sites have low n, reducing confidence in their averages.
Unsampled areas are more uncertain when interpolated, as their values are determined by nearest points.
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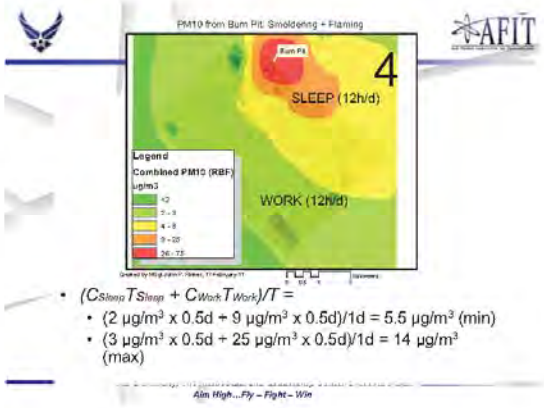
PM10 from Burn Pit: Smoldering + Flaming
Burn Pit
4
SLEEP (12h/d)
WORK (12h/d)
Legend
Combined PM10 (RBF)
µg/m3
• $(C_{Sleep}T_{Sleep} + C_{Work}T_{Work})/T$ =
• (2 µg/m³ x 0.5d + 9 µg/m³ x 0.5d)/1d = 5.5 µg/m³ (min)
• (3 µg/m³ x 0.5d + 25 µg/m³ x 0.5d)/1d = 14 µg/m³ (max)
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Spatial Modeling

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- Gridded sampling with at least 30 sites is recommended
- Georeference all sampling results
- Ideally, should not apply last year's results to this year's deployments
- Sample all season
  - If a person is deployed in the summer (or other season) only, do not use annual averages (and vice versa)
- Sample the same sites
- Synchronous sampling
- Replicate sampling

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Phase I: Small-Scale Emissions Testing

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Small-Scale Testing

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- Funded by AF/SG9
- EPA Open Burn Test Facility, Research Triangle Park NC
- June 2011
- World's foremost experts on air emissions research
- Small-scale testing using a controlled-fire chamber for sampling emissions
- Use simulated deployed waste

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Small-Scale Testing

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1. Types and levels of contaminants (emissions)
   - Dioxins (PXDD, PXDF)
   - CO, CO_2
   - PM10, PM2.5
   - Metals
   - Polycyclic aromatic hydrocarbons (PAHs)
   - Real-time napthalene, BTEX, etc.
2. Resulting emission factors
   - To improve dispersion modeling
3. Address the plastic-no plastic question

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Methodology

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- Burn hut: fire-resistant shed
- Real-time mass (i.e., scale)
- Thermocouples measure burn temperature, room temperature
- Circulation fans
- Make-up air: maintain adequate O_2
- Emissions testing at exhaust & downstream
- Foil-lined to prevent cross-contamination

EPA

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Burn Hut

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Notional Waste Composition

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Plastics (10%)	
PETE	4.5%
HDPE	0.5%
PP	1.5%
PVC	1.0%
PS	1.5%
PU (foams)	0.5%
ABS (electronics)	0.5%
Wood (6%)	
Treated (pallets)	3.0%
Untreated	3.0%
Metals (4.0%)	
Aluminum/Tin	2.0%
Iron/Steel	1.0%
Copper Wire, Insul	1.0%
Misc. Combustibles (75%)	
Fabrics, synthetic	5.0%
Fabrics, natural	10.0%
Canvas, military	2.5%
Cardboard	7.5%
Paper	22.5%
Rubber	2.5%
Wet food waste (slop)	22.5%
Oils and greases	2.5%
Other (5%)	
Glass	3.0%
Building Materials	2.0%

Note: Accelerant fuel (JP-8) is added to ignite solid waste, 1 gal/100 lbs

Assembled Waste

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Burn Hut
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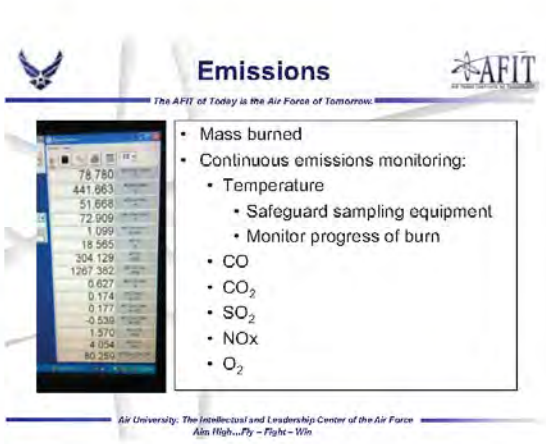
Emissions
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78,780
441,663
51,668
72,909
1,099
18,565
304,129
1267,382
0.627
0.174
0.177
-0.539
1.570
4.054
80.259
• Mass burned
• Continuous emissions monitoring:
• Temperature
• Safeguard sampling equipment
• Monitor progress of burn
• CO
• CO_2
• SO_2
• NOx
• O_2
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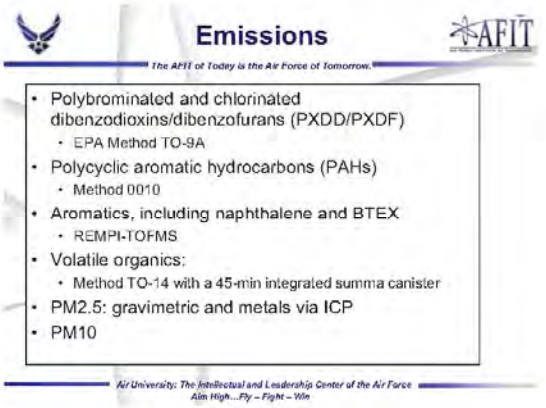
Emissions
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• Polybrominated and chlorinated dibenzodioxins/dibenzofurans (PXDD/PXDF)
• EPA Method TO-9A
• Polycyclic aromatic hydrocarbons (PAHs)
• Method 0010
• Aromatics, including naphthalene and BTEX
• REMPI-TOFMS
• Volatile organics:
• Method TO-14 with a 45-min integrated summa canister
• PM2.5: gravimetric and metals via ICP
• PM10
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Phase II: Large-Scale Emissions Testing

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Large-Scale Emissions Testing

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- AF/SG9 funded
- Burn representative waste at Tooele Army Depot (Tooele UT)

(1) Open burning (surface)
(2) Air curtain burner

- Approximately 10 tons/day (1 roll-off dumpster)
- Emissions testing: near the source
- Downwind measurements
- Sep 2011

Great Salt Lake & Tooele UT

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Large-Scale Testing

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1. Compare emission factors to Phase I
   - What effects do differences in waste composition have on emissions?
     - e.g., city waste vs. constructed waste
   - Can small-scale be used to answer questions in lieu of large-scale burns?
2. Compare emission factors:
   1) Surface burns vs. 2) Burn box
3. Capture downwind samples:
   - To validate software dispersion model
   - To test CO_2 as a surrogate of PM exposure

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2010- Deepwater Horizon

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References

The AFIT of Today is the Air Force of Tomorrow.

1. US Government Accountability Office (GAO), 'Afghanistan and Iraq: DoD Should Improve Adherence to Its Guidance on Open Pit Burning and Solid Waste Management', GAO-11-63, October 2010.
2. Air Force Institute for Operational Health (now USAFSAM), 'Screening Health Risk Assessment Burn Pit Exposures, Balad Air Base, Iraq, and Addendum Report', IOH-RS-BR-TR-2008-0001, May 2008.
3. National Defense Authorization Act (NDAA) for Fiscal Year 2010, Section 317 (prohibition of disposal of certain wastes), October 2009.
4. Department of Defense Directive-Type Memorandum (DTM) 09-032 (in response to NDAA Section 317; prohibition of disposal of certain wastes), March 2010.

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Questions?

Center of the Air Force
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Inhalation Exposure to JP-8 Jet Fuel Enhances Susceptibility to Noise Induced Hearing Loss in Rats

711 HPW/RHPBA

Dr. David Mattie

Studies identified organic solvents as potential ototoxicants promoting noise-induced hearing loss (NIHL). The ability of JP-8 to enhance susceptibility to noise exposure on auditory function was studied in rats. An initial study exposed rats to 0, 75, 85 or 95 dB octave band noise for 6 hours per day, 5 days per week over 4 weeks. Hearing loss was assessed using distortion product otoacoustic emission (DPOAE) to evaluate outer hair cell function and compound action potential (CAP) to determine hearing threshold. Histopathology of cochleas was conducted to determine percentage of hair cell loss. Noise exposure of 85 dB was identified as the LOAEL and was used in the second study to investigate combined effects of JP-8 and noise on hearing by exposing rats to 85 dB and either 0, 200, 750 or 1500 mg/m3 JP-8 for 6 h per day, 5 days per week over 4 weeks. DPOAE, CAP and histopathology of the cochlea for rats exposed to noise and JP-8 showed a dose response increase in hearing loss greater than seen with just 85 dB alone. A third study with just JP-8 alone resulted in no hearing loss indicating JP-8 only potentiates NIHL. A fourth 28-day study consisted of exposures at 102 dB for 15 min per hr for 6 hrs per day, 1000 mg/m3 JP-8 for 6 hr/day, combined exposure to both noise and JP-8, and no experimental treatment. Auditory testing again showed JP-8 by itself didn't produce hearing impairment but male rats were affected more than females.

Inhalation Exposure to JP-8 Jet Fuel Enhances Susceptibility to Noise Induced Hearing Loss in Rats

2 Aug 2011

DAVID R. MATTIE, PhD, DABT
Senior Research Toxicologist
711 HPW/RHPB
Air Force Research Laboratory

Integrity ★ Service ★ Excellence

1

Outline

- Goal and Objectives
- Background
- Methods
  - Basic Design
- Study 1
  - Results
- Study 2
  - Results
- Study 3
  - Results
- Study 4
  - Results
- Future

2

Project Goal and Objectives

- Goal
  - To show if there is an association between jet fuel exposure and noise-induced hearing loss.
    - The hypothesis is that JP-8 jet fuel contributes to hearing loss when combined with high noise exposure that is still below the exposure limit for noise.
- Objectives
  - Design noise generation system for Navy Chambers
  - Conduct 4 28-day animal studies
  - Compare jet fuel exposures and kinetics
  - Publication showing relationship between noise and jet fuel exposure

3

Background

- Dr Fechter exposed rats to 1000 mg/m³ JP-8 for 4 hours followed by either:
  - noise (105 dB) for 4 hours for one day
  - noise (97 dB) for 4 hours; repeated for 5 days
  - noise (102 dB) for 1 hour; repeated for 5 days
- Did not examine multiple dose levels of jet fuel
- Noise level was equivalent to the PEL for noise
  - 90 dB using the A weighting scale for an 8 h TWA
- JP-8 alone did not cause any disruption of auditory function
- Although effects were not consistent combined JP-8 + noise produced greater impairment than noise alone
- JP-8 also caused significant depletion of GSH indicating oxidative stress as a possible mechanism of action for the promotion of hearing loss
- Initial data supporting an interaction between JP-8 and noise exposure on hearing

• Fechter, L.D. Gearhart, C., Fulton, S., Campbell, J. Fisher, J., Na, K., Cocker, D., Nelson Miller, A., Moon, P., and B. Pouyatos. (2007). JP-8 Jet Fuel can Promote Auditory Impairment Resulting from Subsequent Noise Exposure in Rats. Tox. Sci., 96, 510-525

4

Background

- Dr Fechter exposed rats in nose-only chambers
- Subsequent study by his lab showed
  - Acute nose-only exposures caused significant depletion of liver GSH levels after clean, filtered air
  - Same effect seen with JP-8
  - Raises questions about combined JP-8 and noise effects seen in Fechter (2007)
  - Need whole body exposure chambers

- Fechter, L. D., Nelson-Miller, A. and Gearhart, C. (2008). Depletion of Liver Glutathione Levels in Rats: A Potential Confound of Nose-Only Inhalation. Inhalation Toxicology, 20:905–910

AFRL 5

Methods

- Basic Design
  - Using whole body exposure chambers
    - Will eliminate stress that may have contributed to previous findings
  - Noise and JP-8 at the same time
  - Three concentrations of JP-8 plus noise level known to produce mild hearing loss
  - Longer time of exposure and more days of exposure

AFRL 6

Methods

- Major accomplishments:
  - Noise system for inhalation chambers developed by*
    - John Stubbs, Capt, USAF as MS Graduate Student
    - Under mentorship of Lt Col Jeremy Slagley – AFIT Assistant Professor
  - Completed all 4 exposures

*DEVELOPMENT OF A NOVEL NOISE DELIVERY SYSTEM FOR JP-8 OTOTOXICITY STUDIES - THESIS
John E. Stubbs, Captain, USAF
AFIT/GIH/ENV/10-M04
Graduated March 2010

AFRL 7

Methods

- Noise system for inhalation chambers

Shaker mounted on shelf between chamber legs

Audio rack housing system equipment

AFRL 8

Method – Study 1

1. Exposure to noise only
   - Determined level of noise to use in combined noise and JP-8 study
   - Noise exposures in Bldg 837
   - Screen rats for normal hearing (DPOAE testing here)
   - 6 h/day with weekends off for 28 days (20 exp tot)
   - Transport rats to Loma Linda for auditory assessment
   - Study Summary

Group	Exposure Level (dB)	Number of Animals	
		Males	Females
Control	0	5	5
Low	75	5	5
Intermediate	85	5	5
High	95	5	5
Total		20	20

AFRL 9

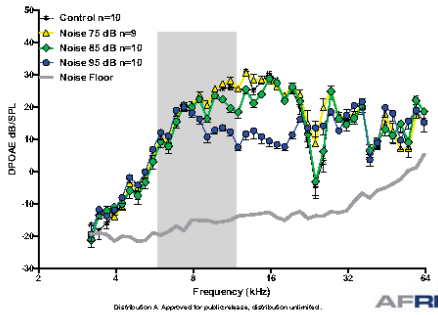
Methods – All 4 Studies

Hearing loss tested by Distortion product otoacoustic emission (DPOAE) test
- Test added as screen for normal hearing prior to each 28-day study
- Assesses hair cell function
- While rats were lightly anaesthetized with ketamine (44 mg/kg) and xylazine (7 mg/kg)
- Both transient and permanent impairments as well as recovery rate
- Intact cochlea is able to generate sound energy when stimulated with two simultaneous tones known as "primary tones" and designated as frequencies "f1" and "f2"
- Sound energy generated by cochlea consists of different frequencies than the "primary tones" so they are "distortion products"
- Possible to detect impairment of hair cells - drop in DPOAE amplitude as a function of length along basilar membrane
- f1 and f2 primaries presented through two separate realistic dual radial horn tweeters (Radio Shack, Tandy Corp., Ft Worth, TX)
- Tones delivered to outer-ear canal through probe, where they acoustically mixed to avoid artifactual distortion
- Ear-canal sound pressure levels were measured by an emissions microphone assembly (Etymotic Research, ER-10Bþ, Elk Grove Village, IL) embedded in the probe

AFRL 10

Results – Study 1: DPOAE

DPOAE 4 Weeks Post Exposure



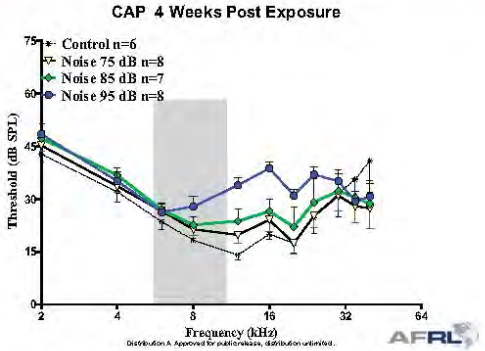
AFRL 11

Methods - All 4 Studies

Hearing loss tested by Compound action potential (CAP) test for hearing threshold
- Performed 4 weeks following the end of exposures by recording compound action potentials (CAPs) from the round window for pure tones between 2 and 40 kHz in approximately ½ octave steps
- Auditory thresholds assessed in a double walled audiometric booth under anaesthetized with xylazine (13 mg/kg, im) and ketamine (87 mg/kg, im)
- Auditory bulla opened via a ventrolateral approach to allow the placement of a fine (od 0.1 mm) Teflon-coated silver wire electrode (A-M Systems, Inc., Carlsborg,WA) onto the round window
- Silver chloride reference electrode was inserted into neck musculature
- Cochlea warmed using a low voltage high-intensity lamp
- CAP signals evoked by pure tones amplified 31000 between 0.1 and 1.0 kHz with a Grass A.C. preamplifier (Model P15, W. Warwick, RI)
- Identified sound level necessary to generate a visually detectable CAP response averaged over four sweeps on a digital oscilloscope (approximate response amplitude of 1 mV measured as the output of the preamplifier)

AFRL 12

Results – Study 1: CAP



AFRL
13

Methods - All 4 Studies

Hearing loss tested by Microscopic examination of the inner ear (cochlea)

- Percentage of receptor loss (outer hair cells - OHCs and inner hair cells - IHCs) in the ear
- After CAP measurements, cochleae harvested
- Within 2 min, cochleae were fixed by perilymphatic perfusion with 1 ml of 3% glutaraldehyde, 2% formaldehyde, 1% acrolein and 2.5% dimethyl sulfoxide in phosphate buffered saline (PBS) pH 7.4)
- Following primary 24-h fixation, tissue was washed with 0.1M PBS, postfixed with 2% OsO_4 in water for 2 h, washed again with 0.1M PBS
- Organ of Corti dissected in 70% ethanol and mounted in glycerin for counting of hair cells
- Cells were counted as present either when stereocilia, cuticular plate or cell nucleus could be visualized
- No attempt to assess degree of possible cellular damage to surviving cells
- Frequency-place map established by Muller (1991) used to superimpose frequency coordinates on length coordinates of organ of Corti
- "map" reflects that cochlea organized tonotopically with high frequency sound producing maximum stimulation of cells in base, and low frequency sound in apex

AFRL
14

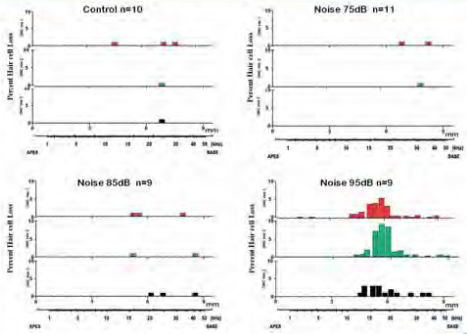
Methods – All 4 Studies

Hearing loss tested by Microscopic examination of the inner ear (cont.)

- Cochleogram showing percentage of hair cell loss as a function of distance from base of cochlea was plotted for each animal
- Results were averaged across each group of subjects for comparison between groups
- Software used for counting cochlear hair cells developed by R. Lataye and Dr. P. Campo from the "Institut National de Recherche et Se´curite´" (Nancy, France)

AFRL
15

Results – Study 1: Cochleogram showing percentage of hair cell loss



AFRL
16

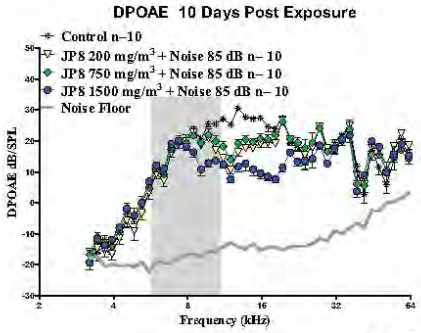
Methods – Study 2

Study 2 - Exposure to noise and JP-8
- Will show combined effects of jet fuel and noise on hearing loss.
- Noise + JP-8 exposures in Bldg 837
- Study initiated 28 Apr 10
- 6 h/day with weekends off for 28 days (20 exp tot)
- Transport rats 1 Jun 10 to Loma Linda for auditory assessment

- 16 rats added for this study only to collect data for target tissue analysis
  - lung, blood, liver, fat, and brain collected at RHPB
  - samples transported to analytical lab
  - Will compare JP-8 exposure data to previous studies

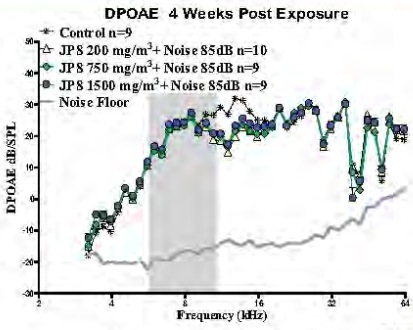
AFRL 17

Results – Study 2 : DPOAE



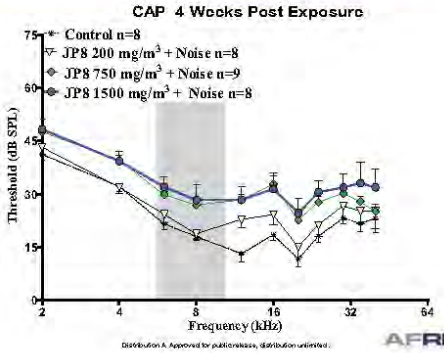
AFRL 18

Results – Study 2 : DPOAE



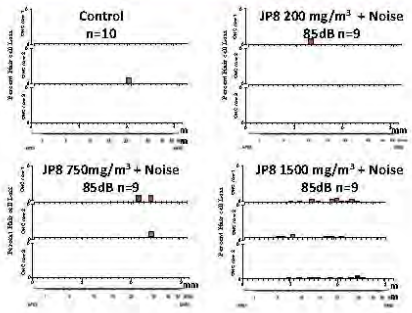
AFRL 19

Results – Study 2: CAP



AFRL 20

Results – Study 2: Cochleogram showing percentage of hair cell loss



AFRL 21

Results – Study 2: Tissue Data

Fat Tissue Analysis – Compounds detected

Methylcyclohexane	1,2,4-Trimethylbenzene
Toluene	Decane
2-Methylheptane	1,2,3-Trimethylbenzene
Octane	Butylcyclohexane
Ethylbenzene	Undecane
m-Xylene	Naphthalene
p-Xylene	Dodecane
o-Xylene	Tridecane
Nonane	Tetradecane
Propylcyclohexane	Pentadecane
1,3,5-Trimethylbenzene	
o-Ethyltoluene	

- Blood, lung, liver, fat & brain tissues analyzed
- Fat tissue showed highest deposition
- Control samples were all nondetect

AFRL 22

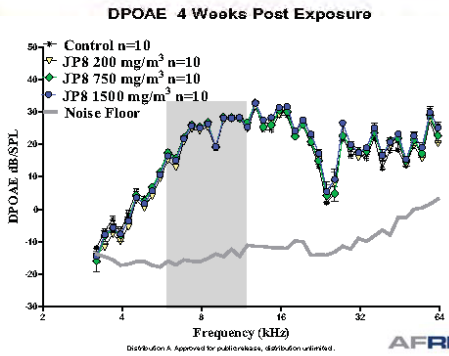
Methods – Study 3

Study 3 - Exposure to JP-8 alone

- Provided data for comparing effects of jet fuel ototoxicity to noise-induced hearing loss in the combined study
- Study design same as Study 2 without noise
  - 5 male rats and 5 female rats per group
  - 0 (control), 200, 750 and 1500 mg/m³ JP-8
  - 6 h/day with weekends off for 28 days (20 exposures total)
  - JP-8 exposures in Bldg 837
  - Transported rats to Loma Linda for auditory assessment
- JP-8 alone did not appear to affect hearing

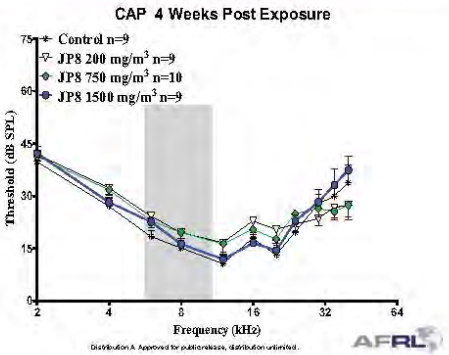
AFRL 23

Results – Study 3: DPOAE

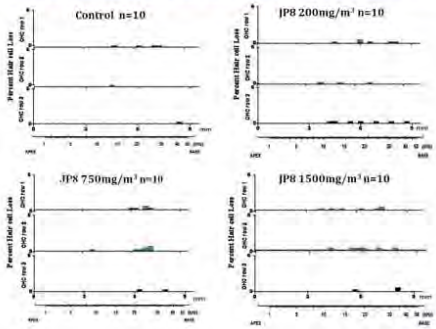


AFRL 24

Results – Study 3: CAP



Results – Study 3: Cochleogram showing percentage of hair cell loss

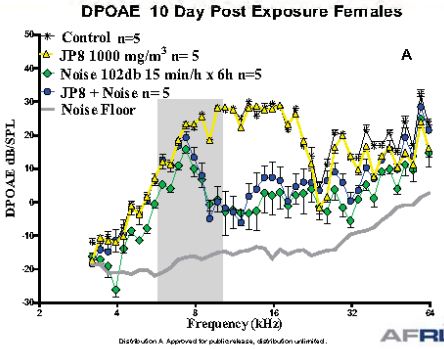


Methods – Study 4

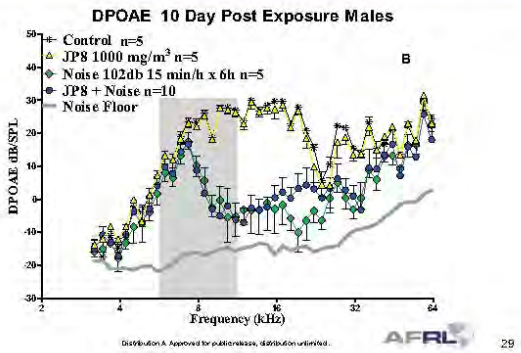
Study 4 - Exposure to a series of intermittent high levels of noise during JP-8 inhalation exposure

- 5 male rats and 5 female rats per chamber
- Design
  - Chamber 1 – air only (Control group for all both treatments)
  - Chamber 2 – JP-8 only (1000 mg/m^3)
  - Chamber 3 – noise at 102 dBA for 15 minutes every hour (1.5 h total noise exposure)
  - Chamber 4 - both JP-8 (same as Chamber 2) and noise (same as Chamber 3)

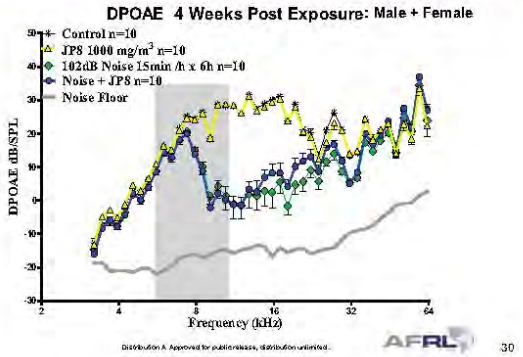
Results – Study 4: DPOAE



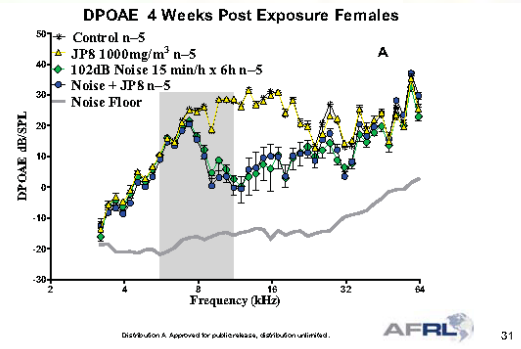
Results – Study 4: DPOAE



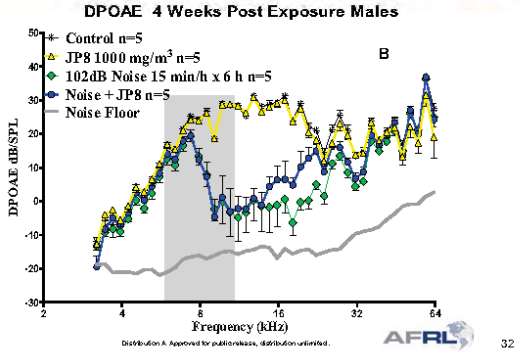
Results – Study 4: DPOAE



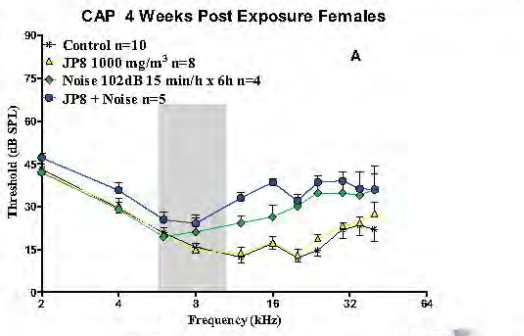
Results – Study 4: DPOAE



Results – Study 4: DPOAE



Results – Study 4: CAP



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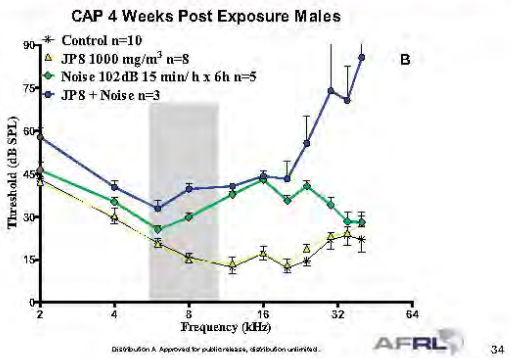
Results – Study 4: CAP



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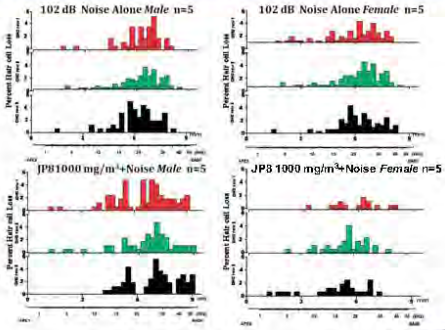
Results – Study 4: Cochleogram showing percentage of hair cell loss



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Future

Future studies

- Use design developed for Study 4
  - Chamber 1 – air only
  - Chamber 2 – fuel only at 1000 mg/m³
  - Chamber 3 – noise only for 6 H/day for 28 days
  - Chamber 4 - both jet fuel (same as Chamber 2) and noise (same as Chamber 3)
- Confirm results and address strain difference by using Long Evans rats
- Study 50:50 mix of F-T alternative fuel and JP-8
- Examine mechanism of action by developing physiologically based kinetic (PBPK) and dynamic (PBPD) models
  - PBPK for kinetics of JP-8 exposure to tissues and cochlea
  - PBPD for development of hearing loss based on noise and jet fuel exposures

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Acknowledgements

Collaborators
- Dr Jeff Fisher - Co-Principal Investigator
  - University of Georgia, Environmental Health Science
  - Now at NCTR, FDA, Arkansas
- Dr Larry Fechter - Co-investigator
  - Loma Linda VA Medical Center
- LT Pedro A. Ortiz, Co-investigator
  - Naval Medical Research Unit-Dayton
- LT Vish Mokashi, Ph.D. - Co-investigator
  - Naval Health Research Center Detachment/Environmental Health Effects Laboratory
  - Now at NRL Detachment RDECOM, Edgewood,MD
- CDR Gail Chapman, Ph.D. - Co-investigator
  - Naval Health Research Center Detachment/Environmental Health Effects Laboratory
  - Now at U.S. Army Medical Research & Materiel Command: Navy Liaison Military Infectious Disease Research Program

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AFRL 37

QUESTIONS?

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AFRL 38

Results – Study 2: Tissue Data

Fat Tissue Analysis (ng/g)

	Dosed Animals							
	9	10	11	12	13	14	15	16
Heptane	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Methylcyclohexane	0.00	90.0*	164.12	0.00	0.00	0.00	0.00	0.00
Toluene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	196.00
2-Methylheptane	0.00	0.00	0.00	0.00	0.00	0.00	248.39	0.00
Octane	0.00	0.00	0.00	0.00	0.00	0.00	175.68	0.00
Ethylbenzene	52.92*	90.60*	110.87	0.00	84.85*	88.98*	84.62*	86.20*
m-Xylene	161.68	151.51	231.26	170.30	176.88	245.88	143.45	158.79
p-Xylene	67.81*	32.21*	63.61*	56.29*	98.33	0.00	61.29*	88.72*
o-Xylene	115.46	114.26	131.94	136.69	153.69	153.01	216.49	143.04
Nonane	0.00	292.75	278.31	79.04*	227.98	299.43	239.03	191.53
Propylcyclohexane	92.30	174.10	90.18	103.45	82.28	131.60	99.22	96.60
1,3,5-Trimethylbenzene	61.71	77.04	42.66	98.26	122.07	150.58	106.27	117.30
o-Ethyltoluene	67.19*	109.00	137.24	89.51*	86.29*	124.43	118.71	121.52
1,2,4-Trimethylbenzene	222.71	197.85	280.15	194.18	258.84	347.74	296.12	273.66
Decane	411.27	429.76	421.75	414.57	395.02	568.09	447.97	580.68
1,2,3-Trimethylbenzene	148.46	121.20	154.65	108.66	136.75	161.42	147.80	169.19
Butylcyclohexane	113.92	119.84	139.18	96.71	102.23	190.24	134.97	168.52
Undecane	800.75	1013.78	1046.93	1033.84	832.11	1457.71	902.72	1167.64
Naphthalene	196.44	123.81	248.32	65.44*	240.11	178.72	162.04	170.06
Dodecane	1287.86	2014.81	2106.69	1950.92	1816.21	2636.59	2147.55	2115.38
Tridecane	1248.58	1849.98	2058.57	1890.62	1743.97	2557.96	2070.97	2144.19
Tetradecane	1897.81	2066.90	2942.46	2186.08	2766.80	2982.48	3694.83	2512.73
Pentadecane	1898.38	2051.32	2049.24	2198.14	3221.06	3078.36	3867.11	2392.22

- Blood, lung, liver, fat & brain tissues analyzed
- Fat tissue showed highest deposition
- Control samples were all nondetect

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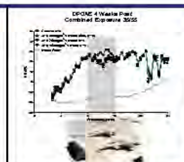
AFRL 39

Evaluation of Jet Fuel Induced Hearing Loss in Rats

Objectives:
- To show if there is an association between jet fuel exposure and noise-induced hearing loss.
- **Hypothesis:** JP-8 jet fuel contributes additively or synergistically to hearing loss when combined with high noise exposure environments, but that would still be below the exposure limit for noise.
- Provide a lab model and a well designed toxicity approach

Description of Effort:
Design a noise generation system for our inhalation chambers and conduct four 28-day animal studies to compare jet fuel and noise exposures as well as kinetics of JP-8.



Benefits of Proposed Technology:
- Hearing loss represents a critical occupational concern; determining fuel exposure as a component required to assure lowest disability formation in Airmen
- Data suggests that Air Force personnel exposed to both jet fuel and noise suffer greater hearing loss than matched Air Force personnel exposed to similar noise but not to jet fuel
- No clear results or definitive studies to show an association between jet fuel exposure and noise
- Lab data relevant to determining need for applying protective measures for jet fuel/noise environments during occupational exposure for Air Force personnel
- Collaboration with Navy Toxicology Division and Veterans Administration Research Laboratory in Loma Linda, CA

Major Goals/Milestones:
- Noise generation system developed by AFIT student for Navy inhalation chambers
- Transitioned DPOAE hearing loss measurement system to Navy in collaboration with Dr. Fechter
- Completed the following 28-day studies – six hours per day, 5 days per week:
- noise-only - 75, 85 or 95 dB
- combined noise (85 dB) and JP-8 jet fuel (3 doses)
- jet fuel alone - 200, 750 or 1500 mg/m^3 JP-8
- only 15 minutes of 102 dBA noise every hour + JP-8
- Poster presented 7 Mar 11 at Soc of Toxicology meeting

Summary of (Present, Preliminary) Results:
JP-8 plus noise showed a dose response increase in hearing loss greater than seen with just noise alone.

Distribution A. Approved for public release, distribution unlimited.

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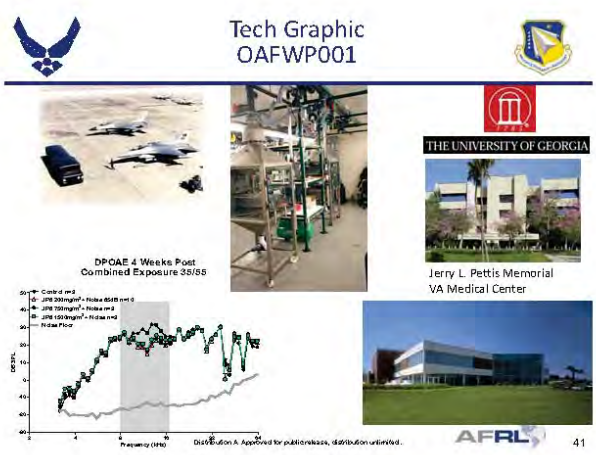
Tech Graphic
OAFWP001
THE UNIVERSITY OF GEORGIA
DPOAE 4 Weeks Post
Combined Exposure 35/85
Jerry L. Pettis Memorial
VA Medical Center
AFRL
41

Assessing Operationally Relevant Aspects of Nanoparticle Exposure Health Risks

711 HPW/USAFSAM-PHT

Dr. Clarise Starr

There is little known in the scientific literature regarding the potential dangers and downstream sequelae caused by exposure to nanoparticles. This lack of information has led to conjecture about the potential uses and dangers associated with this new technology, including the possibility that nanoparticles could be used as a weapon to target the warfighter. The purpose of this effort is to answer basic, previously untested parameters regarding nanomaterials to assess the relevance to the potential exposure (from both modified and unmodified nanomaterials) in the field. Three commercial grade nanoparticles--ZnO, TiO2, and CeO2, were studied for personal protective equipment (PPE) efficiency, initial uptake by cell lines, and downstream cytotoxic effects. Preliminary data suggest PPE provided good barriers against nanoparticle exposure. Initial exposure to nanoparticles (2 hr) showed an interaction with the cells, but uptake of the nanoparticles varied depending on cell line. The nanoparticles that were found to be cytotoxic had a longer exposure to the cell lines, indicating that long-term exposure may be key to overall health risks.

Assessing Operationally Relevant Aspects of Nanoparticle Exposure Health Risks

Clarise Rivera Starr, Ph.D.
USAFSAM/FHT
Wright-Patterson AFB, OH

Every Airman a Force Multiplier
August 2011 AFMS Research Symposium

Nanotechnology

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Uses of Nanomaterials

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Everyday Uses	"Biological" Uses
Eyeglasses	Targeted drug delivery
Crack-resistant paints	Targeted gene therapy
Transparent sunscreens	Anti-microbial coatings
Stain-repellent fabrics	Fluorescent labeling
Self-cleaning windows	Imaging contrast
Solar cells for solar panels	Tissue engineering
Automotive	DNA probes
Planes	Microsurgical techniques

The Dark Side to Nano

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The Dark Side to Nano

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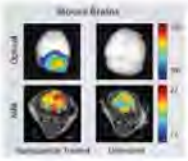
Lung Exposure



Crossing the Blood Brain Barrier

5

Project Overview

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ZnO	TiO_2	CeO_2

Early intake, uptake (early)

Incubation with various cell types

Microscopy (SEM, TEM, etc.)

Cytotoxicity (late)

ROS and cell viability experiments

Lung co-culture cellular models

Ability to detect/protect

PPE and particle counter evaluations

6

Early Uptake/Intake Model

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- Neuroblastomas, macrophages, Hep-G2, and primary trach/bronchial cells
  - Received from ATCC; grown to their specifications
  - Low passage numbers (<p20), then seeded overnight
  - Grown to 80% confluency before challenged with NP
- Initial nanoparticle (NP) concentration ranges from 10 mg/mL-0.1 mg/mL (w/v, resuspended in culture media)
  - 10 mg/mL "suffocated" the cells; CPEs visualized after 2 h
  - 0.1 mg/mL working concentration; allowed to incubate at 37 °C for 2 hs before microscopy performed
- Microscopy performed by UTSA Dept. of Physics
  - SEM (Scanning Electron Microscopy)
  - STEM (Scanning Transmission Electron Microscopy)
  - EDX (Energy Dispersive X-ray)
  - LABE (Low Angle Backscattered Electron)

7

Neuroblastomas

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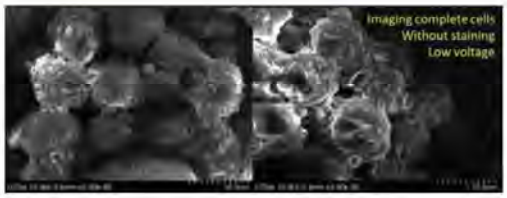

8

CeO_2 Attaches to Cell Membrane with Some Internalization

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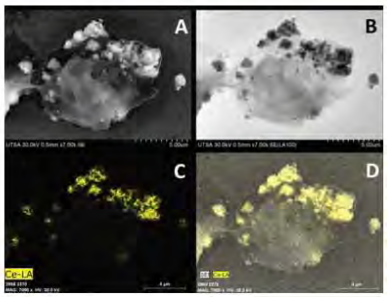

CeO_2—Thin Sections

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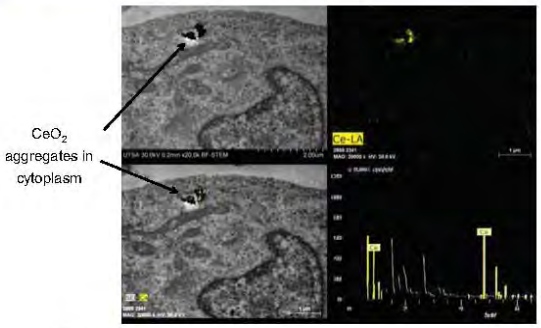

ZnO Is Readily Absorbed into the Cells and Shows Changes in Cell Morphology

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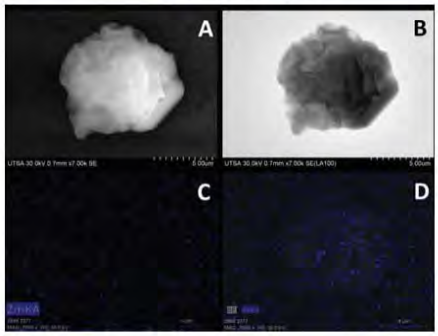

ZnO—Thin Sections

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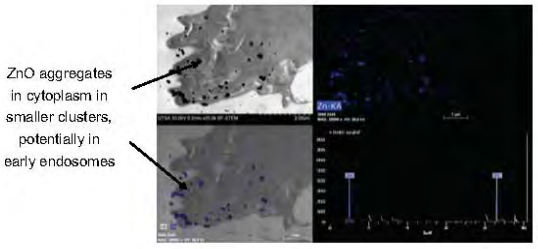

TiO_2 Nanoparticles Are Internalized and Show Changes in Cell Morphology

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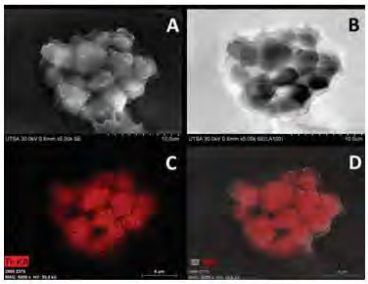

Kipen et al. 2002. Curr Micro 44:49-53

TiO_2—Thin Sections

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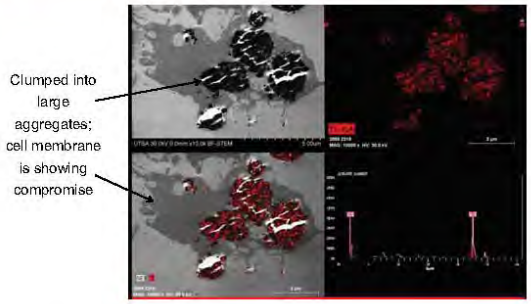

Cell Viability—HaCaT Cells

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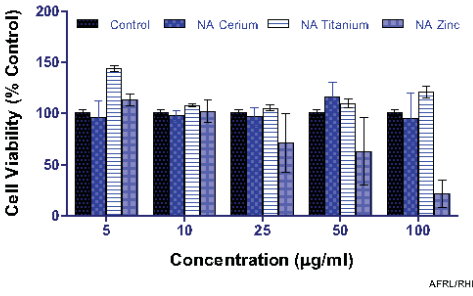

Cell Viability—Lung Co-Culture Model

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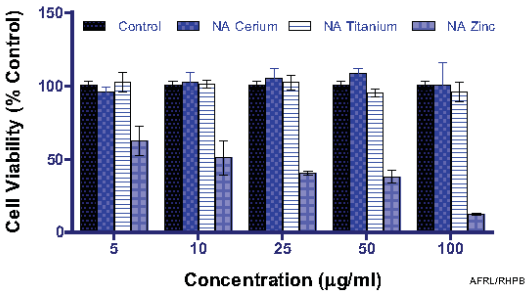

Generation of Reactive Oxygen Species—Lung Co-Culture Model

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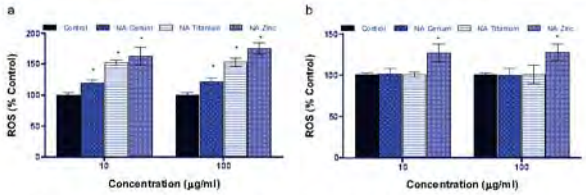

Generation of ROS in lung co-culture model after exposure to various nanoactive nanoparticles. (a) 1 h. All 3 of the NA NPs show generation of significant amounts of ROS. (b) 24 h. Only the NA Zinc NPs showed significant amounts of ROS. This indicated that the cells were able to recover after exposure to the NA Cerium and the NA Titanium Dioxide but not the NA Zinc.

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PPE and Aerosolized Detection

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U of Iowa

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Two XRF Instruments Agree Fairly Well (Iowa data taken with USAF XRF device)

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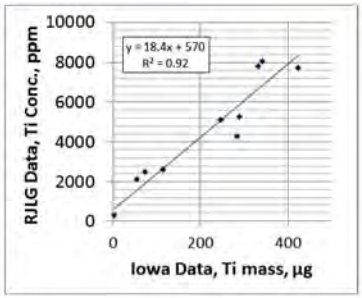

 19

PPE Studies

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Research was performed on the collection efficiency of filters of a new mask. The collection efficiency is high (near 100%) for all particle sizes and types tested.

 20

Conclusions

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- 3 types of NP were examined for the uptake/intake of cells, the cytotoxicity effects, and the ability to detect NP and protect the warfighter in accidental or intentional exposure.
- CeO_2 was not readily taken up by neuroblastoma cells, attaching itself to the cell membrane and aggregating in small clumps in the cytoplasm when it did cross over.
- TiO_2 was readily taken up by neuroblastoma cells and aggregated in very large clumps in the cell, affecting the integrity of the cell membrane.
- ZnO was readily taken up by the neuroblastoma cells and was extremely dispersed into the cell.

Conclusions (cont.)

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- ZnO was extremely cytotoxic (dose dependent) in the cell viability models tested, while CeO_2 and TiO_2 produced little or no cytotoxicity to the cell.
- NP generated ROS after 12 h; however, ZnO continued producing ROS after 24 h, while the other NP-exposed cells were able to recover.
- XRF instrumentation was able to detect all 3 NP that were tested down with good sensitivity.
- Studies of the filters in a new mask showed high collection efficiency against all NP tested.

Way Forward

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- Continue work on other cell types to determine if uptake is different for these cell types.
- Evaluate models for long-term exposure.
- Evaluate TiO_2 at several time points 2-24 h to determine if clustering of NPs resolves itself in the cell and if the data will match the cytotoxicity data collected to date.
- Continue to evaluate particle counters and PPE to ensure that they are able to protect against NP exposure.

Acknowledgments

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Dr. Tom Peters
Kristin Bunker
Gary Cassucio
Bruce Pacolay

AFRL/RHPB
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25

Transport of Silver Nanoparticles in Saturated Porous Media: Experimental Results and Model Simulations

AFIT/ENV

Capt Jason Flory

Nanosilver is the largest and fastest growing category of nanomaterial, with extensive USAF and DoD applications. A growing number of studies show that nanosilver may pose significant adverse human and environmental effects. Given the ubiquity of nanosilver and its potential toxicity, it is incumbent upon us to understand its environmental fate and transport. Due to the importance of groundwater as a pathway from contamination sources to human and environmental receptors, this study examined how nanosilver is transported in saturated porous media. In the study, silver nanoparticles (AgNPs) were synthesized in the laboratory using a sodium borohydride reduction method. The transport of these nanoparticles in a saturated porous media packed column was investigated. Both a conservative tracer and AgNPs were injected into water flowing through the laboratory column (diameter: 2.5 cm, length: 15 cm) packed with water-saturated quartz sand to obtain concentration-versus-time breakthrough curves. The AgNPs were found to break through before the conservative tracer, perhaps due to the facilitated transport of AgNPs (i.e., AgNPs moved through larger pores, and therefore moved faster than the tracer). It was also observed that the total mass of AgNPs leaving the column was smaller than the total input mass, indicating the capture of a fraction of the colloidal AgNPs by the porous media. Filtration theory was used to simulate the transport behavior of the AgNPs in the quartz sand packed column.


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Transport of Silver Nanoparticles in Saturated Porous Media: Experimental Results and Model Simulations
U.S. AIR FORCE
Capt Jason Flory
Bioenvironmental Engineer
AFIT/ENV
2 August 2011
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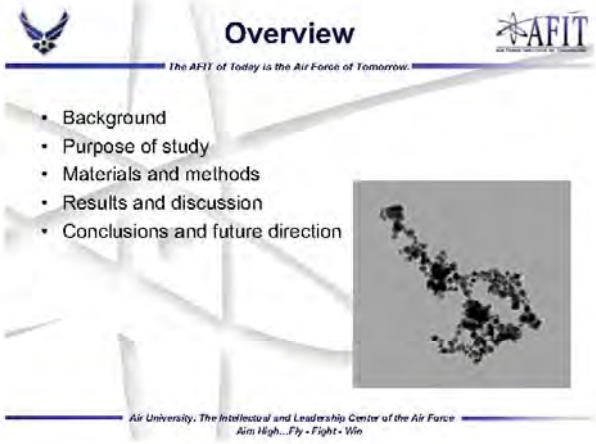
Overview
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Background
Purpose of study
Materials and methods
Results and discussion
Conclusions and future direction
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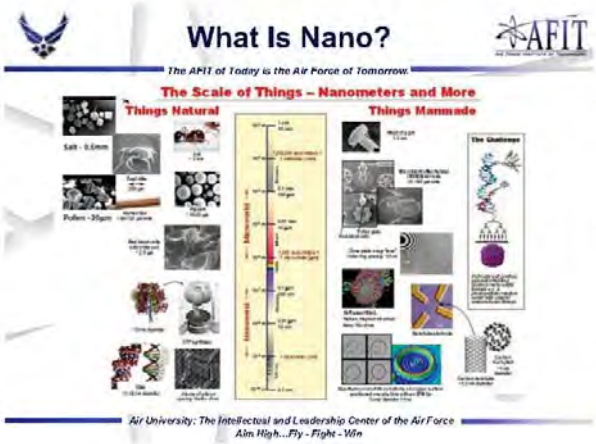
What Is Nano?
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The Scale of Things – Nanometers and More
Things Natural
Things Manmade
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Commercial Applications
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Nanosilver in Footwear
Antibacterial Nanosilver Infused in Storage Containers
Uses of Ag NPs
Nanosilver and Antimicrobial Personal Care
Nanosilver Coated Surfaces of Medical Devices to Reduce Hospital Related Infections
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Military Applications

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Aerospace:
- High-strength, low-weight composite materials
- Improved electronics
- Physical sensors
- Air purification technology

Obscurants

Antimicrobial Agents:
- Purification Filters
- Wound dressings
- Clothing

Radar System:

Nano-enhanced Weapon System

Nanosuits

Sunscreens

Nanoenergetics:
- cost/space efficient fuels

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Nanosilver In Environment

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- Fish toxicity studies suggest hazards unique to nanoscale
- Naturally occurring silver in water is typically colloidal/nanoscale
- As particle size shrinks, there is tendency for toxicity to increase, even if same material is relatively inert in bulk form
  - Ag NPs have demonstrated greater potential to travel through organ systems compared to larger materials
  - May not be detected by phagocytic defenses, allowing them to gain access to blood or cross blood–brain barrier into nervous system
- Environmental conditions affect stability/persistence
  - pH
  - Ionic strength

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Purpose Of Study

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- Investigate how AgNP is transported in groundwater under environmental conditions
  - pH
  - Ionic strength
- Compare/contrast transport of AgNP, Ag+
- Clarify transport process
  - Understanding of risks from releases
  - Better decision-making to manage releases

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Column Experiments

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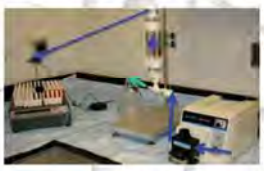
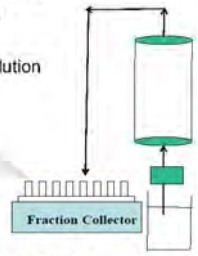
- Glass columns, packed with glass beads, fed by peristaltic pump with AgNP suspensions
  - Background solution: 0.01 mM KCl
  - Flow rate: 1 mL/min
  - Vary pH, ionic strength of AgNP solution





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SEM of quartz sand porous media
Size of pores in sand = nm to micron; this study simulates sand with glass beads
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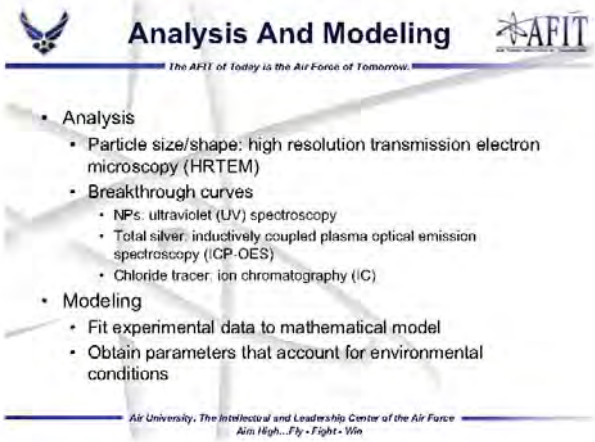
Analysis And Modeling
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Analysis
Particle size/shape: high resolution transmission electron microscopy (HRTEM)
Breakthrough curves
NPs: ultraviolet (UV) spectroscopy
Total silver: inductively coupled plasma optical emission spectroscopy (ICP-OES)
Chloride tracer: ion chromatography (IC)
Modeling
Fit experimental data to mathematical model
Obtain parameters that account for environmental conditions
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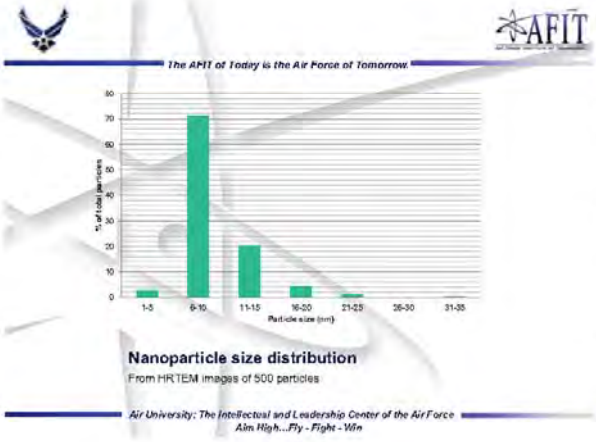
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Nanoparticle size distribution
From HRTEM images of 500 particles
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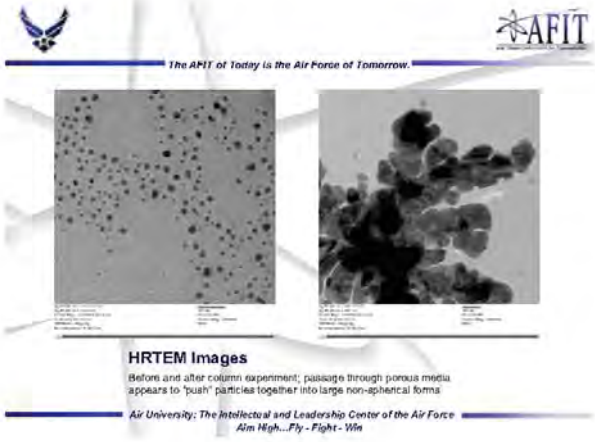
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HRTEM Images
Before and after column experiment; passage through porous media appears to "push" particles together into large non-spherical forms
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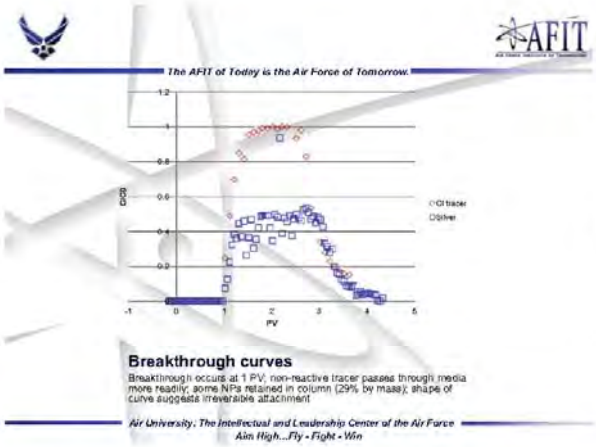
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AFIT
C/C0
PV
Cl tracer
Other
Breakthrough curves
Breakthrough occurs at 1 PV; non-reactive tracer passes through media more readily; some NPs retained in column (29% by mass); shape of curve suggests irreversible attachment
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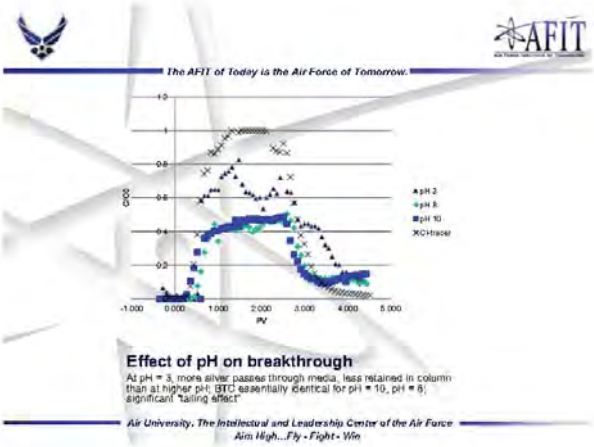
The AFIT of Today is the Air Force of Tomorrow.
AFIT
C/C0
PV
pH 3
pH 8
pH 10
Cl tracer
Effect of pH on breakthrough
At pH = 3, more silver passes through media, less retained in column than at higher pH; BTC essentially identical for pH = 10, pH = 8; significant "tailing effect"
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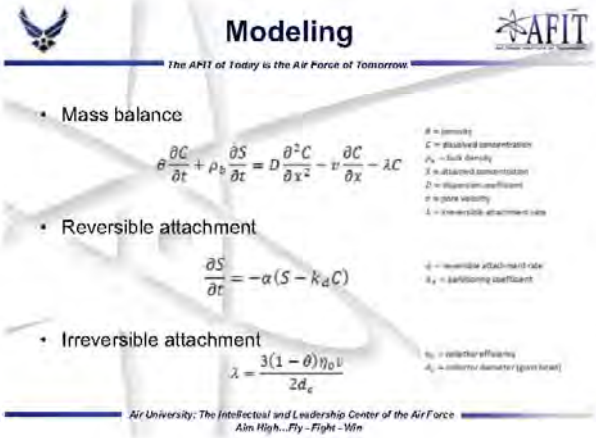
Modeling
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AFIT
Mass balance
$\theta\frac{\partial C}{\partial t}+\rho_b\frac{\partial S}{\partial t}=D\frac{\partial^2 C}{\partial x^2}-v\frac{\partial C}{\partial x}-\lambda C$
Reversible attachment
$\frac{\partial S}{\partial t}=-\alpha(S-k_d C)$
Irreversible attachment
$\lambda=\frac{3(1-\theta)\eta_0 v}{2d_c}$
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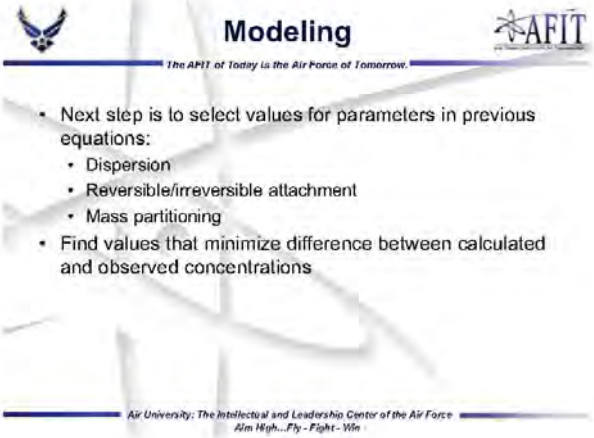
Modeling
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AFIT
Next step is to select values for parameters in previous equations:
Dispersion
Reversible/irreversible attachment
Mass partitioning
Find values that minimize difference between calculated and observed concentrations
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Future Direction

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- This study:
  - Repeat, confirm experimental results
  - Conduct similar experiments, varying ionic strength at constant pH
  - Examine attachment difference between AgNP, Ag^+
  - Continue modeling process, finding parameters to account for environmental effects
- Future studies:
  - Explore other media types: sand, soil
  - Refine model: account for effects of flow rate, media properties

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Acknowledgements

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- Wright State University
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  - Jessica Dagher
- Funding support from AFMSA/SG9S Intramural Studies Program

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Questions?

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- Background
- Purpose of study
- Materials and methods
- Results and discussion
- Conclusions and future direction

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References

The AFIT of Today is the Air Force of Tomorrow.

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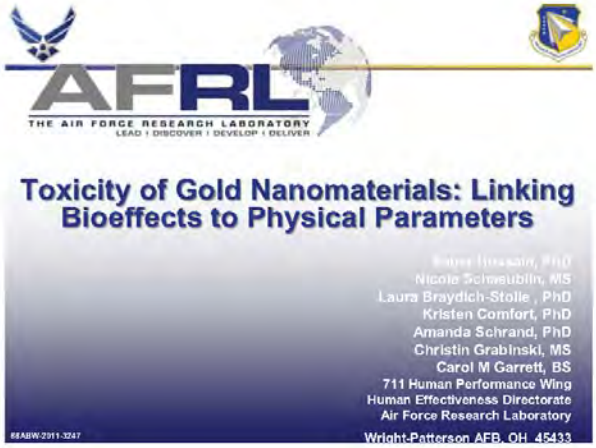
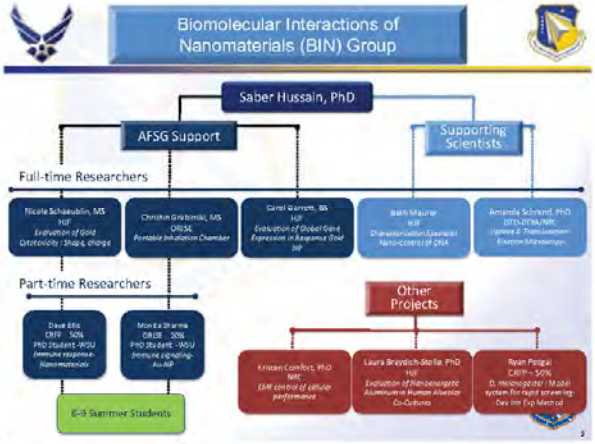
Evaluation of Gold Nanomaterial Toxicity Based on Physical and Chemical Properties

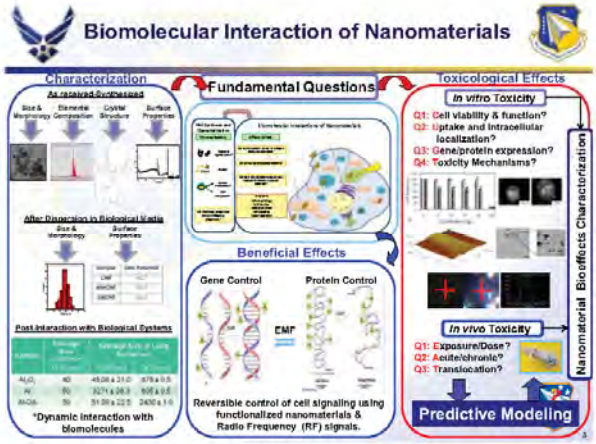
711HPW/RHPBA

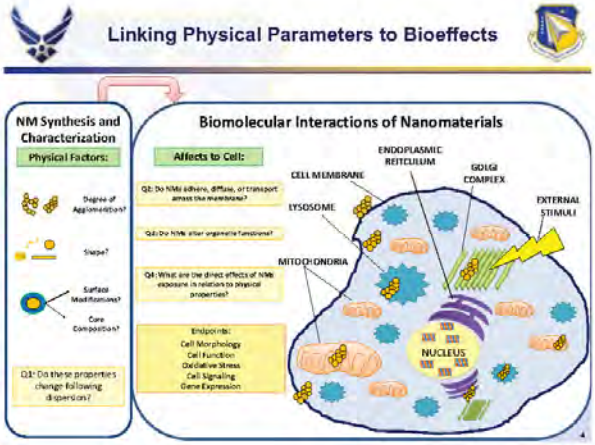
Dr. Saber Hussain

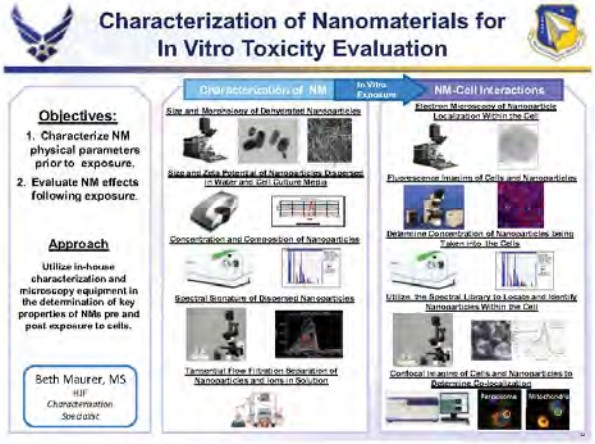
Gold nanomaterials (Au NMs) have distinctive electronic and optical properties, making them ideal candidates for biological, medical and defense applications. Therefore, it is important to evaluate the potential biological impact of Au NMs before employing them in any application. In the present study, we investigated whether the size, charge and shape of the Au NMs plays a role in mediating a biological response in an in vitro model of human skin cells. The results demonstrated that smaller 0.8nm and 1.5nm Au NP's were toxic in a concentration dependent manner, regardless of charge. However, gene expression studies showed that the 1.5nm Au NPs induced DNA damage and down-regulated the DNA repair mechanism with these genes varying based on charge. Further, the results have illustrated that the gold nanorods (17nm AuNR-PEG (AR=2.1)) were cytotoxic to the skin cells, while the gold nanospheres (20nm AuNS-MPS) were not toxic even at the highest dose of 100 µg/ml. Additionally, exposure to the 17nm AuNR-PEG (AR=2.1) caused the formation of significant amounts of ROS, and the up-regulation of several genes involved in cellular stress and toxicity. In summary, these results indicated that size, surface charge, and shape play a key role in mediating the cellular response to Au NMs.

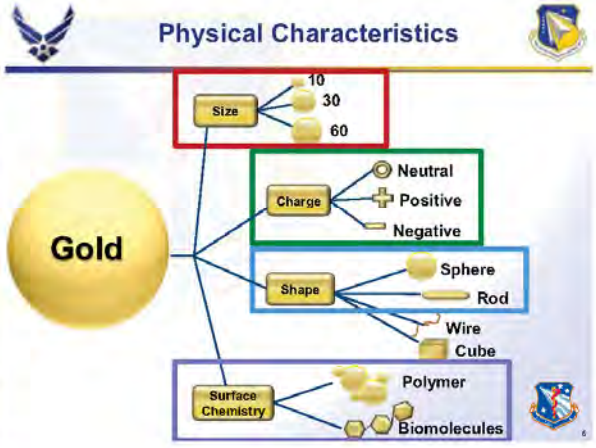

AFRL
THE AIR FORCE RESEARCH LABORATORY
LEAD • DISCOVER • DEVELOP • DELIVER
Toxicity of Gold Nanomaterials: Linking Bioeffects to Physical Parameters
Saber Hussain, PhD
Nicole Schaeublin, MS
Laura Braydich-Stolle , PhD
Kristen Comfort, PhD
Amanda Schrand, PhD
Christin Grabinski, MS
Carol M Garrett, BS
711 Human Performance Wing
Human Effectiveness Directorate
Air Force Research Laboratory
Wright-Patterson AFB, OH 45433
88ABW-2011-3247

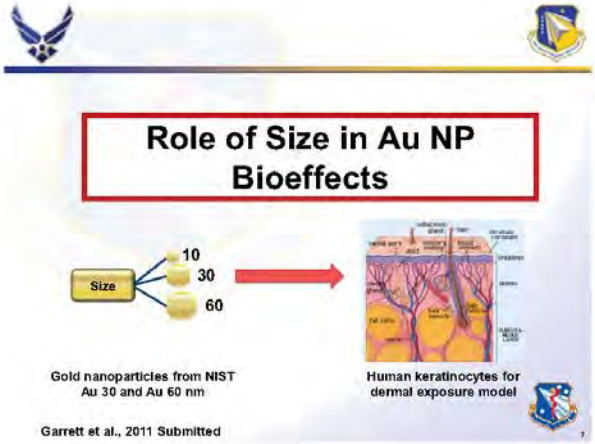

Biomolecular Interactions of Nanomaterials (BIN) Group
Saber Hussain, PhD
AFSG Support
Supporting Scientists
Full-time Researchers
Part-time Researchers
Other Projects
8-9 Summer Students

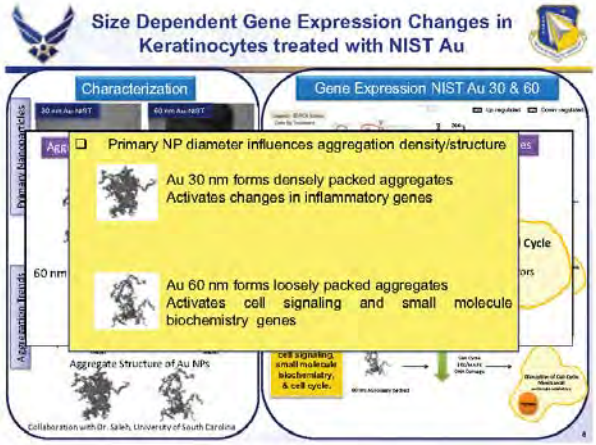

Biomolecular Interaction of Nanomaterials
Characterization
As received/ Synthesized
After Dispersion in Biological Media
Post-interaction with Biological Systems
*Dynamic interaction with biomolecules
Fundamental Questions
Beneficial Effects
Gene Control
Protein Control
EMF
Reversible control of cell signaling using functionalized nanomaterials & Radio Frequency (RF) signals.
Toxicological Effects
In vitro Toxicity
Q1: Cell viability & function?
Q2: Uptake and intracellular localization?
Q3: Gene/protein expression?
Q4: Toxicity Mechanisms?
In vivo Toxicity
Q1: Exposure/Dose?
Q2: Acute/chronic?
Q3: Translocation?
Predictive Modeling
Nanomaterial Bioeffects Characterization

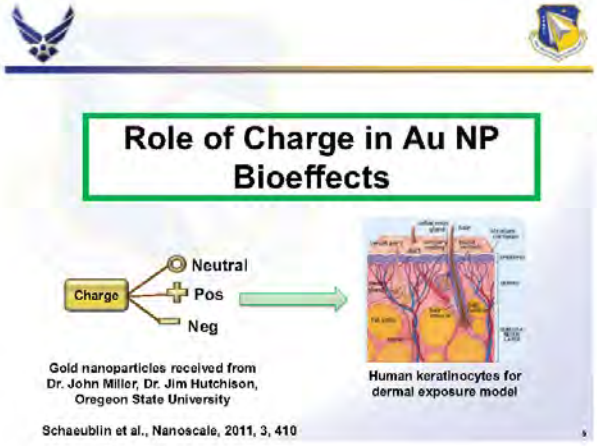

Linking Physical Parameters to Bioeffects
NM Synthesis and Characterization
Physical Factors:
Biomolecular Interactions of Nanomaterials
Affects to Cell:
CELL MEMBRANE
ENDOPLASMIC RETICULUM
GOLGI COMPLEX
LYSOSOME
EXTERNAL STIMULI
MITOCHONDRIA
NUCLEUS
Endpoints:
Cell Morphology
Cell Function
Oxidative Stress
Cell Signaling
Gene Expression

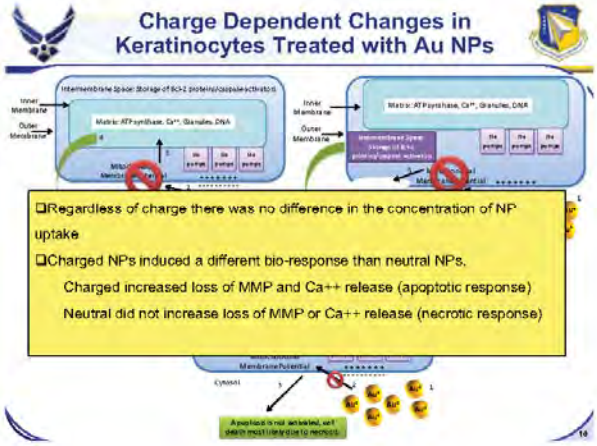

Characterization of Nanomaterials for In Vitro Toxicity Evaluation
Objectives:
1. Characterize NM physical parameters prior to exposure.
2. Evaluate NM effects following exposure.
Approach
Utilize in-house characterization and microscopy equipment in the determination of key properties of NMs pre and post exposure to cells.
Beth Maurer, MS
Characterization Specialist
Characterization of NM
In Vitro Exposure
NM-Cell Interactions
Size and Morphology of Dehydrated Nanoparticles
Size and Zeta Potential of Nanoparticles Dispersed in Water and Cell Culture Media
Concentration and Composition of Nanoparticles
Spectral Signature of Dispersed Nanoparticles
Tangential Flow Filtration Separation of Nanoparticles and Ions in Solution
Electron Microscopy of Nanoparticle Localization Within the Cell
Fluorescence Imaging of Cells and Nanoparticles
Determine Concentration of Nanoparticles being Taken into the Cells
Utilize the Spectral Library to Locate and Identify Nanoparticles Within the Cell
Confocal Imaging of Cells and Nanoparticles to Determine Colocalization
Peroxisome
Mitochondria

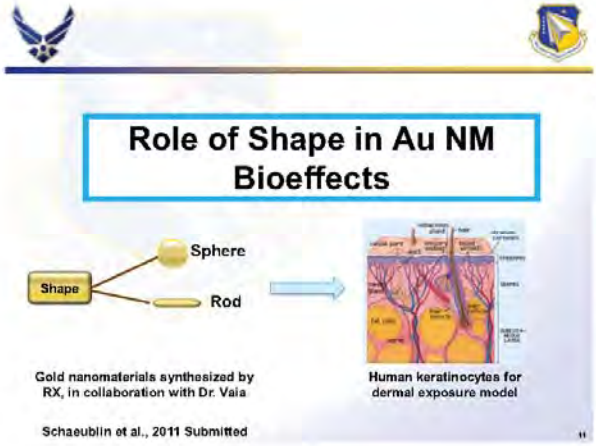

Physical Characteristics
Gold
Size
10
30
60
Charge
Neutral
Positive
Negative
Shape
Sphere
Rod
Wire
Cube
Surface Chemistry
Polymer
Biomolecules

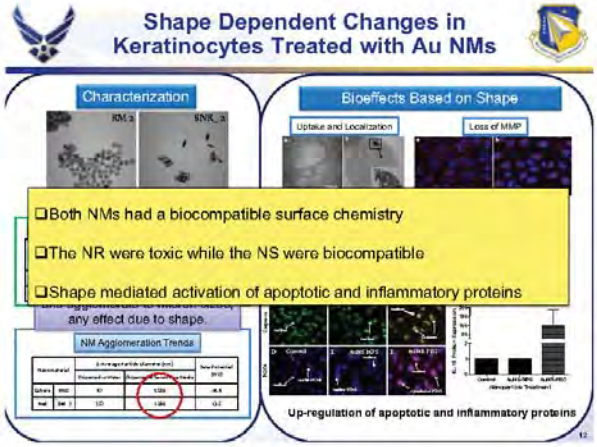

Role of Size in Au NP Bioeffects
Size
10
30
60
Gold nanoparticles from NIST Au 30 and Au 60 nm
Human keratinocytes for dermal exposure model
Garrett et al., 2011 Submitted

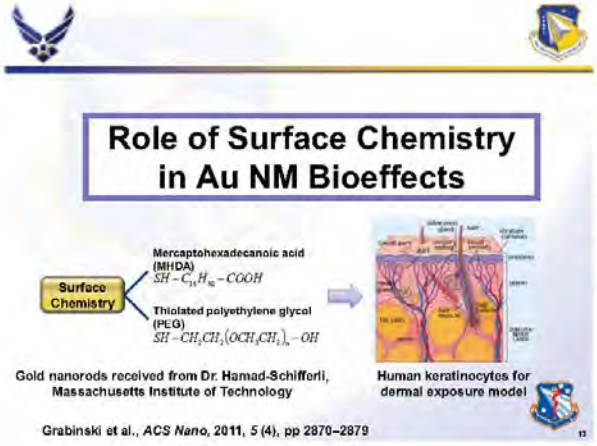

Size Dependent Gene Expression Changes in Keratinocytes treated with NIST Au
Characterization
Gene Expression NIST Au 30 & 60
Primary NP diameter influences aggregation density/structure
Au 30 nm forms densely packed aggregates
Activates changes in inflammatory genes
Au 60 nm forms loosely packed aggregates
Activates cell signaling and small molecule biochemistry genes
60 nm
Aggregate Structure of Au NPs
Cycle

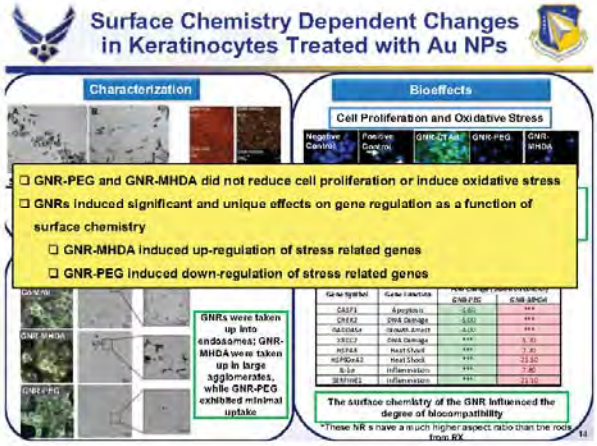

Role of Charge in Au NP Bioeffects
Charge
Neutral
Pos
Neg
Gold nanoparticles received from Dr. John Miller, Dr. Jim Hutchison, Oregeon State University
Human keratinocytes for dermal exposure model
Schaeublin et al., Nanoscale, 2011, 3, 410

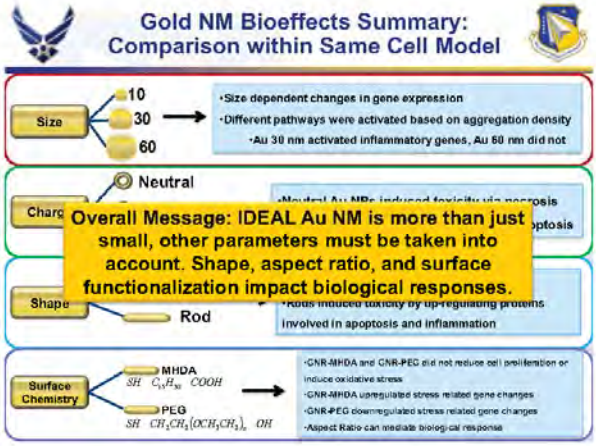

Charge Dependent Changes in Keratinocytes Treated with Au NPs
Regardless of charge there was no difference in the concentration of NP uptake
Charged NPs induced a different bio-response than neutral NPs.
Charged increased loss of MMP and Ca++ release (apoptotic response)
Neutral did not increase loss of MMP or Ca++ release (necrotic response)

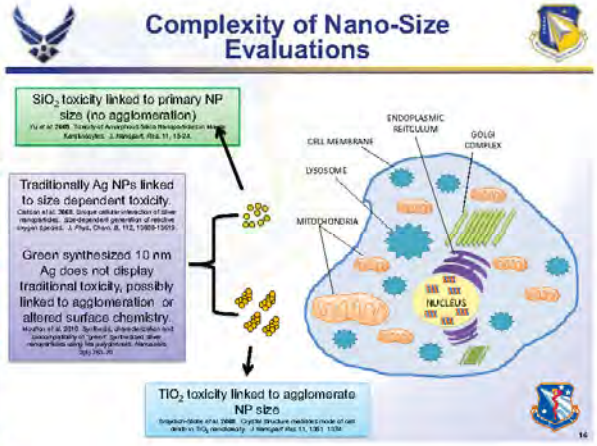

Role of Shape in Au NM Bioeffects
Shape
Sphere
Rod
Gold nanomaterials synthesized by RX, in collaboration with Dr. Vaia
Human keratinocytes for dermal exposure model
Schaeublin et al., 2011 Submitted

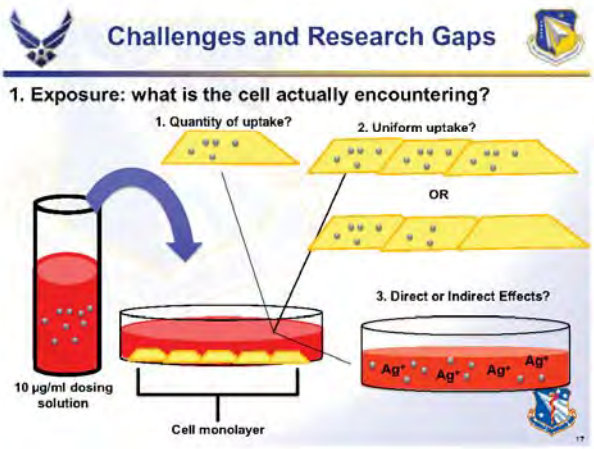

Shape Dependent Changes in Keratinocytes Treated with Au NMs
Characterization
Bioeffects Based on Shape
Uptake and Localization
Loss of MMP
Both NMs had a biocompatible surface chemistry
The NR were toxic while the NS were biocompatible
Shape mediated activation of apoptotic and inflammatory proteins
any effect due to shape.
NM Agglomeration Trends
Up-regulation of apoptotic and inflammatory proteins

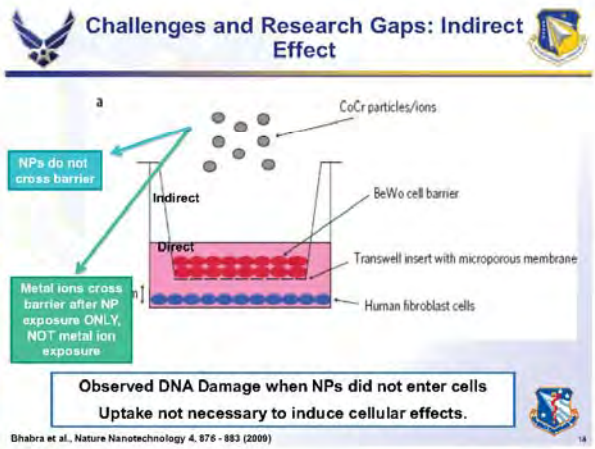

Role of Surface Chemistry in Au NM Bioeffects
Surface Chemistry
Mercaptohexadecanoic acid (MHDA)
$SH-C_{15}H_{30}-COOH$
Thiolated polyethylene glycol (PEG)
$SH-CH_2CH_2(OCH_2CH_2)_n-OH$
Gold nanorods received from Dr. Hamad-Schifferli, Massachusetts Institute of Technology
Human keratinocytes for dermal exposure model
Grabinski et al., ACS Nano, 2011, 5 (4), pp 2870–2879

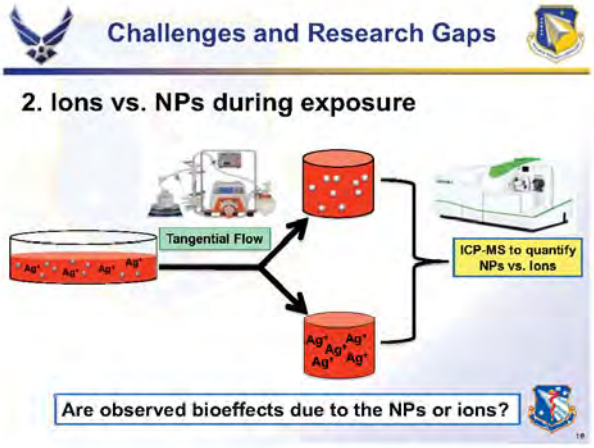

Surface Chemistry Dependent Changes in Keratinocytes Treated with Au NPs
Characterization
Bioeffects
Cell Proliferation and Oxidative Stress
GNR-PEG and GNR-MHDA did not reduce cell proliferation or induce oxidative stress
GNRs induced significant and unique effects on gene regulation as a function of surface chemistry
GNR-MHDA induced up-regulation of stress related genes
GNR-PEG induced down-regulation of stress related genes
GNRs were taken up into endosomes; GNR-MHDA were taken up in large agglomerates, while GNR-PEG exhibited minimal uptake
The surface chemistry of the GNR influenced the degree of biocompatibility

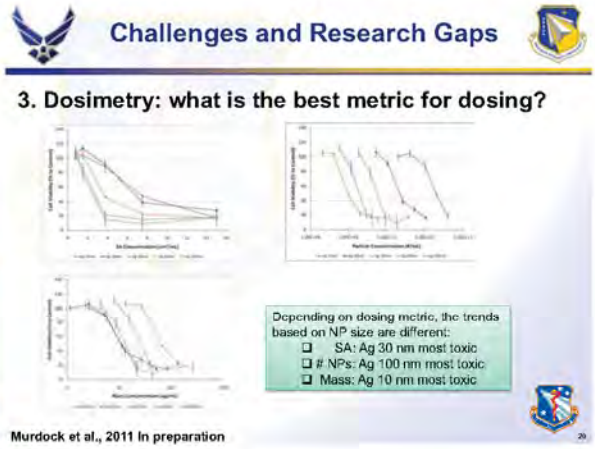

Gold NM Bioeffects Summary: Comparison within Same Cell Model
Size
10
30
60
Size dependent changes in gene expression
Different pathways were activated based on aggregation density
Au 30 nm activated inflammatory genes, Au 60 nm did not
Neutral
Shape
Rod
Rods induced toxicity by up-regulating proteins involved in apoptosis and inflammation
Surface Chemistry
MHDA
PEG
GNR-MHDA and GNR-PEG did not reduce cell proliferation or induce oxidative stress
GNR-MHDA upregulated stress related gene changes
GNR-PEG downregulated stress related gene changes
Aspect Ratio can mediate biological response
Overall Message: IDEAL Au NM is more than just small, other parameters must be taken into account. Shape, aspect ratio, and surface functionalization impact biological responses.


Complexity of Nano-Size Evaluations
SiO_2 toxicity linked to primary NP size (no agglomeration)
Traditionally Ag NPs linked to size dependent toxicity.
Green synthesized 10 nm Ag does not display traditional toxicity, possibly linked to agglomeration or altered surface chemistry.
TiO_2 toxicity linked to agglomerate NP size
ENDOPLASMIC RETICULUM
GOLGI COMPLEX
CELL MEMBRANE
LYSOSOME
MITOCHONDRIA
NUCLEUS


Challenges and Research Gaps
1. Exposure: what is the cell actually encountering?
1. Quantity of uptake?
2. Uniform uptake?
OR
3. Direct or Indirect Effects?
10 µg/ml dosing solution
Cell monolayer
Ag+


Challenges and Research Gaps: Indirect Effect
a
CoCr particles/ions
NPs do not cross barrier
Indirect
Direct
BeWo cell barrier
Transwell insert with microporous membrane
Human fibroblast cells
Metal ions cross barrier after NP exposure ONLY, NOT metal ion exposure
Observed DNA Damage when NPs did not enter cells
Uptake not necessary to induce cellular effects.
Bhabra et al., Nature Nanotechnology 4, 876 - 883 (2009)


Challenges and Research Gaps
2. Ions vs. NPs during exposure
Tangential Flow
ICP-MS to quantify NPs vs. Ions
Ag+
Are observed bioeffects due to the NPs or ions?


Challenges and Research Gaps
3. Dosimetry: what is the best metric for dosing?
Depending on dosing metric, the trends based on NP size are different:
SA: Ag 30 nm most toxic
NPs: Ag 100 nm most toxic
Mass: Ag 10 nm most toxic
Murdock et al., 2011 In preparation

Challenges and Research Gaps

4. *In vitro* vs. *In vivo*?

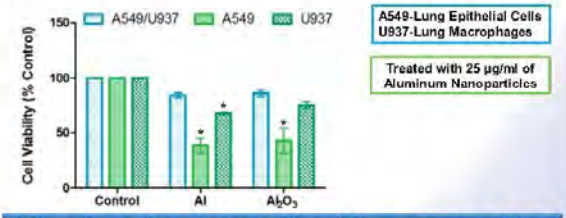
•Cell monolayer does not represent true complexity of a tissue/organ

- **Animal models**
  - Accurate
  - Similar to human
  - Time consuming
  - Costly
  - Ethical issue
- ***In vitro* models**
  - Approximate estimation & predictive
  - Rapid (reduce time)
  - Less expensive

At the earliest stages of toxicity screening, when least is known about the new molecule, simple *in vitro* models with end points revealing a general sense of toxicity are appropriate to aid in making decisions

Challenges and Research Gaps

Co-cultures: A better approach to *in vitro* work



When the cells were co-cultured the presence of the immune cells protected the epithelial cells
More realistic of *in vivo* scenario!

Braydich-Stolle et al., *ACS Nano*, 2010, *4* (7), pp 3661-3670

Overall Message?

- **Size matters, but not always:**
  - Primary vs. Agglomerated "Critical point"
  - Monodispersed vs. Polydispersed

Nanotoxicology Requires Characterization since Bioeffects are Directly Linked to Physical Parameters!

- **Other Physical Characteristics Matter:**
  - Shape
  - Charge
  - Coating

Contribute to chemical reactivity and observed bioeffects!

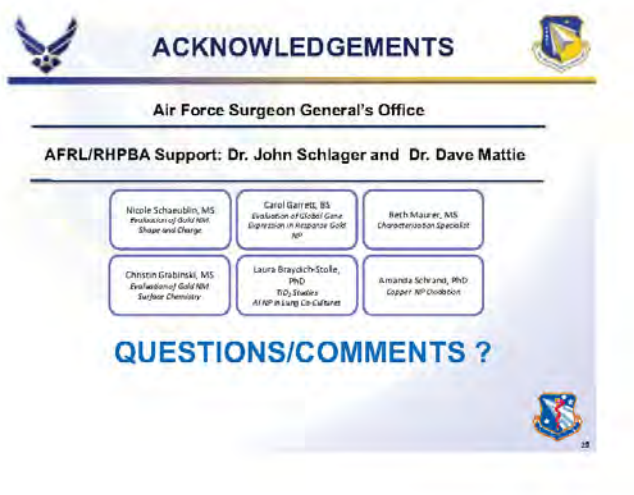
Editorial Highlight in Toxicological Sciences IMPACT

TOXICOLOGICAL HIGHLIGHT

How Meaningful are the Results of Nanotoxicity Studies in the Absence of Adequate Material Characterization?

Associate Editor of Tox.Sci.

Murdock RC, Braydich-Stolle L, Schrand AM, Schlager JJ and Hussain SM (2007) Characterization of nanomaterial dispersion in solution prior to in vitro exposure using dynamic light scattering technique. Toxicol Sci 101:239-253.


ACKNOWLEDGEMENTS
Air Force Surgeon General's Office
AFRL/RHPBA Support: Dr. John Schlager and Dr. Dave Mattie
Nicole Schaeublin, MS
Carol Garrett, BS
Beth Maurer, MS
Christin Grabinski, MS
Laura Braydich-Stolle, PhD
Amanda Schrand, PhD
QUESTIONS/COMMENTS ?

Nanomaterial Hazard Identification: The Zebrafish Model for Rapid Material Testing

349th Medical Squadron (349 MDS)

Maj Joseph Fisher

Force Health Protection is facing a new challenge both in-garrison and in deployed operations as the nanotechnology revolution begins. The National Science Foundation predicts the period from 2011-2020 will result in fundamentally new products based on nanomaterials. These chemical biophysical nanometer scale (i.e., 1 x 10-9 meters) materials may bring new or increased hazard to humans and the environment, and the uncertainty surrounding their risk to biological and environmental health needs to be investigated. Health risk can be defined as a function of hazard and exposure, and an understanding of the hazard and exposure of these materials is important in order to minimize health risk. Products utilizing nanoscale materials will become ubiquitous throughout commerce in the coming years and regulatory oversight and reporting in the EU and the US is moving forward. The development of the zebrafish (Danio rerio) model for rapid material testing bridges a gap in toxicology testing between in vitro cell culture models and in vivo mammalian models. The anatomy, physiology, and genomics of the zebrafish are highly homologous to humans, and these similarities are just beginning to be exploited by research communities. Being a whole animal vertebrate organism, zebrafish allow for great flexibility in conducting experimental assays to identify nanomaterial exposure effects in morphology, physiology, behavior, and distribution. This research presents an overview of the issues surrounding nanomaterial health risk and provides testing results in order to demonstrate the utility of the zebrafish model in answering nanomaterial bio-compatibility research questions.

Nanomaterial Hazard Identification: The Zebrafish Model for Rapid Material Testing

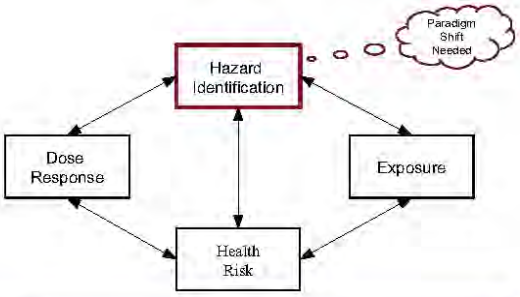
Joseph A Fisher, Maj, USAFR, BSC
Robert L Tanguay, PhD
Oregon State University

Integrity - Service - Excellence

Overview

- Introduction
- Nanomaterials
- Zebrafish (*Danio rerio*)
- Hazard Identification
- Automation
- Testing Results
- Conclusions
- Acknowledgment

Introduction



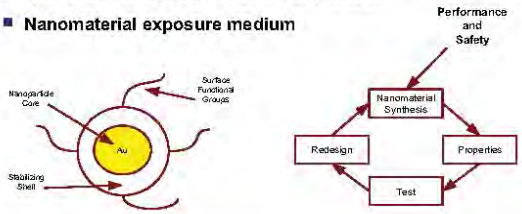
Introduction

- Testing Platforms
  - In silico (virtual screening, models)
  - In vitro (primary/finite and continuous cell cultures)
  - In vivo (whole animals study):
    - Mouse / rat
    - Fish / amphibian
    - Fly / worm - invertebrate
  - Clinical trials
  - Epidemiology

Nanomaterials

Nanomaterial Hazard Identification
Zebrafish

- Nanomaterial types, synthesis, and functionalization
- Nanomaterial detection and measurement
- Nanomaterial exposure medium



5

Nanomaterials

Nanomaterial Hazard Identification
Zebrafish

Gold Nanoparticles and Surface Functionalization
Precisely engineered – well characterized materials

Core Size	0.8 nm	1.5 nm
# Au atoms	11	101
# Ligands per NP	10	30-55
Molecular weight (g)	3,527	24,382
Surface area (sq nm/NP)	12.6	22.9

Ligand	Charge
THAT - N,N,N-trimethylammoniumethanethiol	cationic (positive)
MES - 2-mercaptoethanesulfonate	anionic (negative)
MEEE - (mercaptoethoxy(ethoxy))ethanol	neutral

Agglomeration
Dissolution
Environmental Interactions
Environmental Fate
Biological Interactions
Biological Fate
Biological Responses

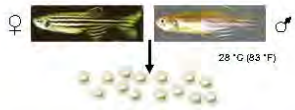
Goal: Predict effect from nanomaterial properties

6

Zebrafish (Danio rerio)

Nanomaterial Hazard Identification
Zebrafish

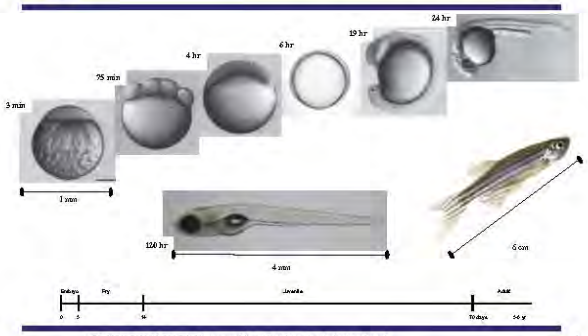
- History (1822 to present)
- Development (embryo, fry, juvenile, adult)
- Anatomy and Physiology
- Genomics (Zv9 assembly)



7

Zebrafish (Danio rerio)

Nanomaterial Hazard Identification
Zebrafish



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Hazard Identification

Nanomaterial Hazard Identification
Zebrafish

- **Strengths**
  - Higher throughput and more information at a lower cost
  - Fast translucent ex utero embryo development
  - Homologous to vertebrates and humans and a sequenced genome
- **Weaknesses**
  - Methods, assays, and tests in development
  - Not a mammal, little in vivo nanomaterial data to compare to
- **Opportunities**
  - Guide development of nanoscience
  - Develop rapid relevant platforms to collect "response" data
  - Identify physiochemical properties that drive biological response
  - Investigate development, disease, regeneration, and human science

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Hazard Identification

Nanomaterial Hazard Identification
Zebrafish

- **Accessing Physiochemical Biological Response**
  In Vivo – Testing whole organism
  - **Tier 1: Toxicity Screening**
    - **Morphology, physiology, behavior assays**
  - **Tier 2: Cellular Targets and Distribution**
    - **Cell death assay**
    - **Distribution assay**
  - **Tier 3: Molecular Expression**
    - **Gene expression assay**

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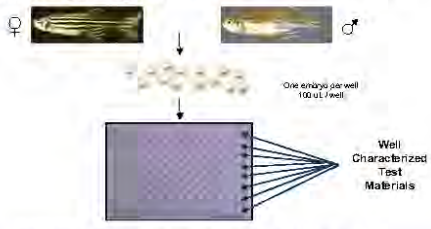
Hazard Identification

Nanomaterial Hazard Identification
Zebrafish

- **Tier 1 Testing – Morphology, physiology, behavior**
  In Vivo – Testing whole organism



Screen for responses (effect) during 1-5 days post fertilization (dpf)

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Hazard Identification

Nanomaterial Hazard Identification
Zebrafish

- **Tier 1 Effect Assessment – between 24 and 120 hpf**
  In Vivo – Testing whole organism

  - **Morphology**
    snout, jaw, brain, eye, otic, edema, notochord, somite, fin (pectoral, caudal), axis, trunk
  - **Physiology**
    heart rate, circulation
  - **Behavior**
    spontaneous movement – onset / frequency
    touch response – head / tail
    swimming response – light / dark / tap

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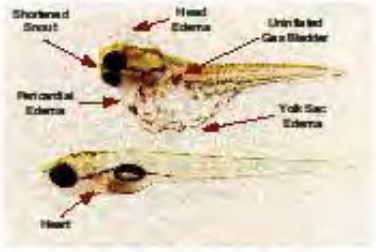
Hazard Identification

- Tier 1: Accessing Morphology

In Vivo – Testing whole organism



Test Material

Control

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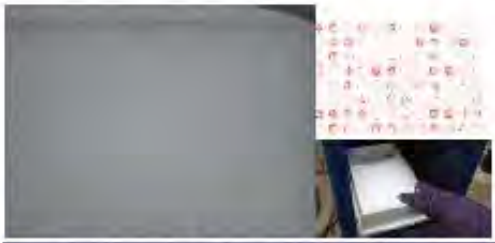
Hazard Identification

- Tier 1: Accessing Behavior

In Vivo – Testing whole organism



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Automation

- Embryo production
- Embryo dechorination
- Exposure plating (media, embryo)
- Assay
  - Time lapse imaging / detection

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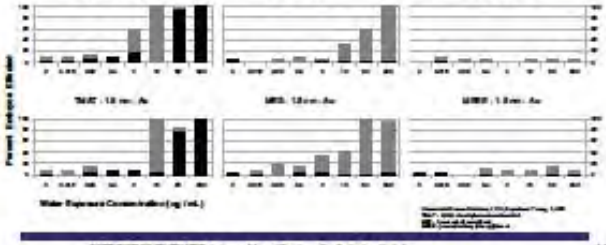
Testing Results

Morphology



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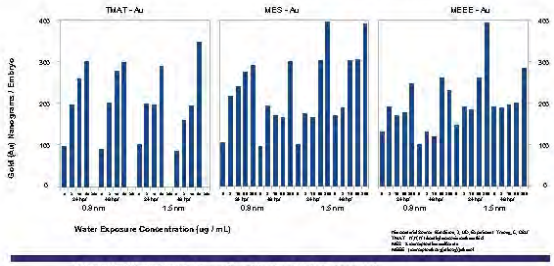
Testing Results

Nanomaterial Hazard Identification
Zebrafish

Distribution (uptake)

Nanomaterial uptake at 24 and 48 hpf – 3 ligand types and 2 sizes
Statically exposed to gold (Au) nanomaterials from 6 hpf

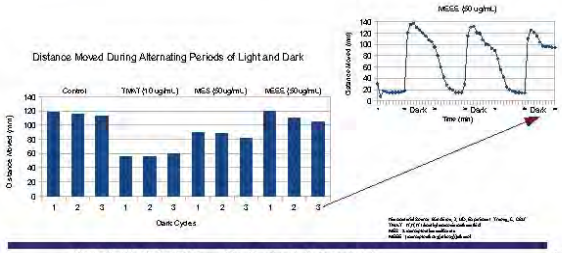


Testing Results

Nanomaterial Hazard Identification
Zebrafish

Behavior

Zebrafish movement at 120 hpf – 3 ligand types at 1.5 nm
Statically exposed to gold (Au) nanomaterials from 6 hpf



Conclusions

Nanomaterial Hazard Identification
Zebrafish

Zebrafish (*Danio rerio*)

- **Robust in vivo model organism platform to evaluate nanomaterial biological interactions**
- **Vertebrate animal homologous to humans, sequenced genome, sensitive at multiple levels**
- **Compatible with high throughput screening, automation, pathway, and mechanistic studies**
- **The NANO revolution has begun – get ready**

Acknowledgment

Nanomaterial Hazard Identification
Zebrafish

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Nanomaterial Hazard Identification
Zebrafish
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Carrie Barton
Leah Wehmas
Lisa Truong
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Galen Miller
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Britton Goodale
Jill Franzosa
Joe Fisher
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EPA RD-833320
NIEHS P3000210, ES016896
Nanomaterials
Hutchison, J; Zaikova, T; University of Oregon; Gold
Orr, G; Pacific Northwest National Laboratory; Silica
Naik, R; Air Force Research Laboratory; Quantum Dot
Experiment
Nanomaterial Source: Hutchison, J, UO
Experiment: Truong, L, OSU
TMAT N,N,N-trimethylammoniumethanethiol
MHS 2-mercaptoethanesulfonate
MSBS 1-[(mercaptoethoxy)ethoxy]ethanol
Working Groups
National Cancer Institute (NCI) Biomedical Informatics Grid (caBIG) Nanotechnology
Office of the Secretary of Defense (OSD) Nanomaterials ESOH
The Oregon Nanoscience and Microtechnologies Institute (ONAMI)
Safer Nanomaterials and Nanomanufacturing Initiative (SNNI)
SNNI
ONAMI
OSU
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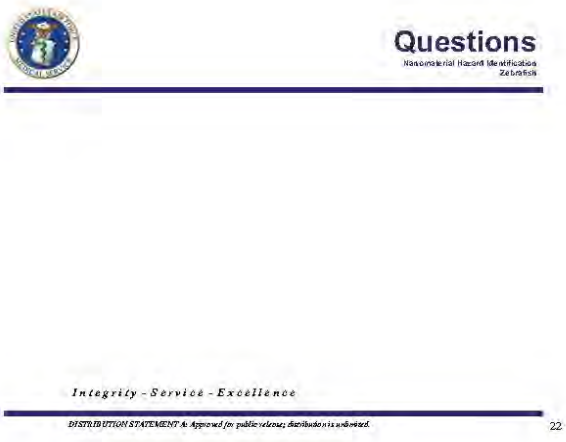
Questions
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Zebrafish
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USAF Efficient Running: An Integrated Program To Reduce Running Injury and Improve Individual Performance in USAF Fitness Assessment

AFMSA/SG6

Lt Col Antonio Eppolito (presented by Lt Col (ret) Dan Kulund)

Running is an essential duty in the USAF "Fit to Fight" culture. Its importance is more critical now as the USAF Fitness Assessment (FA) will have more emphasis on the aerobic component, now 60% of the score, and more frequent testing. Because of this mandate (ref. AFI 36-2905), running has risen to the #2 cause of recreational injuries in the USAF (ref. Descriptive Epidemiology USAF Lost Workday Injuries 2008 report). The annual FA failure rate has doubled from 10% to 20% with the new PFT standards. (As high as 28% at some bases) And yet, the USAF lacks an evidence and experience based program specifically for running which is clear, simple, and understandable and can be incorporated into standardized training for all troops. There are huge direct costs to the military for running injuries and poor FA performances: (1) Medical and Physical Therapy treatment of injuries (clinic visits, MRI's, x-rays, therapy, etc) with a resultant backlog of sports medicine orthopedic referrals of up to 6 months at many MTFs (2) Cost of compensation to AD, ANG and USAFR members who are "injured" while running during duty time and cannot perform their job (3) Costs of command directed programs for retraining annual FA failures and wasted administrative time for retesting, profiles, and waivers (4) Missed work time due to injuries and appointments (5) Needless generation of preventable MEBs. There are also indirect costs which may be even greater: (1) Early separation due to low FA performance scores and failures (2) Decreased productivity due to lack of fitness and overall good health (concept of presenteeism) (3) Deteriorating morale (4) Permanent disability. Injury-free daily aerobic activity supports optimal physical wellness, mental clarity, weight management, and reduces health care utilization. Evidence-based training tools are applied to almost all skills of such importance and most athletic activities except for running. Furthermore, where they are applied most methods are traditional, inefficient, and not standardized. The 2008 USAF Lost Workdays Report highlights the emergence of running injuries and recommends immediate implementation of preventive strategies to address all aspects of running including; injury prevention countermeasures, volume of training, focused lower extremity strengthening and flexibility, proper gait technique and proper footwear. "Efficient Running" is in direct alignment with all the corrective strategies outlined in the critical report and provides the countermeasures. Efficient Running then is our proposed solution. It is based on the biomechanical principles of the most revolutionary concept in the arena of sports medicine in 40 years. It addresses injury prevention and performance improvement and is grounded in scientific principle and extensive real world experience of over 15 years. Efficient Running is a set of training tools to prevent injury and improve efficiency/performance. Our approach involves teaching and tailoring aerobic principles, putting the body in proper alignment, improving running gait biomechanics, and supplementing with essential core strength, balance and dynamic stability exercises.

Headquarters U.S. Air Force

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USAF Efficient Running

Lt Col Antonio Eppolito, USAF, MC
Lt Col (Dr.) Mark Cucuzzella, AFRC
Lt Col (ret) Dan Kulund,

3 Aug 11

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Air Force Running

- Our physical fitness needs improvement
- We expected to be required to sustain the standards required in basic training
- ...living in tent cities
- ...working on flight lines in extreme heat
- ...called upon to defend the base
- Issue PT gear
- January 2004 is the date. Be ready.

RUNNING NOW BIG PART OF AIR FORCE LIFE

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Running Injuries

RUNNING INJURIES SECOND ONLY TO BASKETBALL

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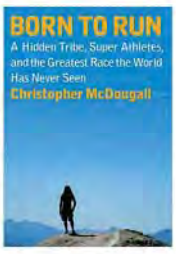
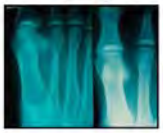
Natural Running





"BORN TO MOVE"

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Form Training
ChiRunning
Evolution Running
good form running
VOLODALEN
STRIDE MECHANICS
POSE TECH
natural running
Barefootrunning
NEWTON
FORM TRAINING PROGRAMS ABOUND
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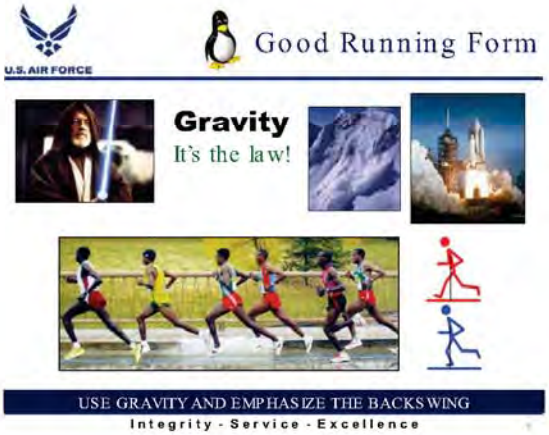
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Good Running Form
Gravity
It's the law!
USE GRAVITY AND EMPHASIZE THE BACKSWING
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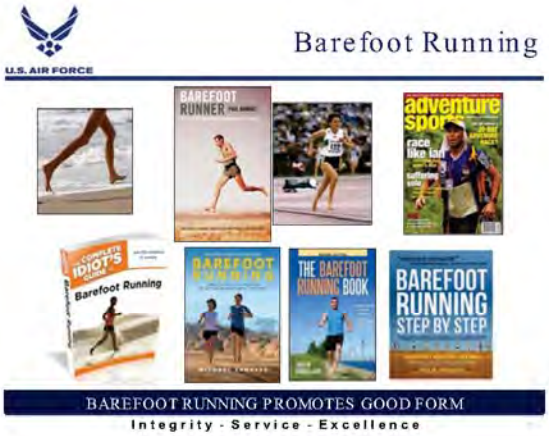
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Barefoot Running
BAREFOOT RUNNER
adventure sports
Barefoot Running
THE BAREFOOT RUNNING BOOK
BAREFOOT RUNNING STEP BY STEP
BAREFOOT RUNNING PROMOTES GOOD FORM
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Running Shoes
Swiss Army shoe
UK Gear
Vibram FiveFingers
Saucony Kinvara Racing Flat
MINIMALIST FOOTWEAR MAKING HEADLINES
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The Future
FLATTER FOOTWEAR FAVORED FOR THE FUTURE
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Preparatory Phase
Chi Running survey
Run Softly trial
New Trends in Running Injury Prevention
Natural Running website
Efficient Running Working Group
Eight form workshops
Medical Corps Examiner articles
Building training modules
BUILD SCIENTIFIC PLATFORM FOR EFFICIENT RUNNING
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UVA SPEED Clinic
LOW IMPACT EASILY TRAINED
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Runner Maintenance Clinic
AMX
RMX
SYSTEMATIC EVALUATION IN RMX CLINIC
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Dynamic Analysis
OBSERVE HOW RUNNER MOVES
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Home Treatment Program
HOME TREATMENT PUTS RUNNERS IN CONTROL
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The Injured Runner
Anteromedial plica
Trochanteric knot
UNFORTUNATE ANATOMY
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U.S. AIR FORCE
The Injured Runner
Soleus band
Calcaneal cliff
UNFORTUNATE ANATOMY
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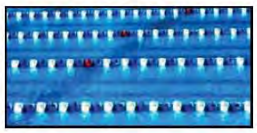
Deep Water Running



ISOKINETIC ENDURANCE TRAINING

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Preparing For Running

Ankles

Hip drops

Toe lifts

SIMPLE PREPARATION

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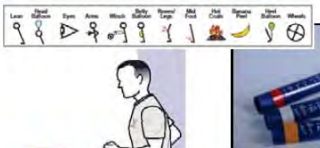
Basic Aerobic Training Tool



BATT CUES GOOD RUNNING FORM

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Return-to-Running

Step	Walk	Run	Reps	Time
1	5 minutes	1 minute	5	30 minutes
2	4	2	5	30
3	3	3	5	30
4	2	4	5	30
5	1	5	5	30

STEPWISE RETURN-TO-RUNNING

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Interval Training

Week	Speed	Recovery	Monday	Wednesday	Friday
1	Fast 30 seconds	Slow 30 seconds	6 repeats	15-20 minute easy run	8
2	Fast 30 seconds	Slow 30 seconds	6	same	10
3	Fast 45 seconds	Slow 45 seconds	6	same	8
4	Fast 45 seconds	Slow 45 seconds	6	same	10
5	Fast 60 seconds	Slow 60 seconds	6	same	8
6	Fast 60 seconds	Slow 60 seconds	6	same	10

Citizen Airman Magazine

STRATEGY TO REDUCE RUNNING TIME
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Operational Phase

- Deploy strategies from Phase I
- Air Force Telehealth generates modules
- Briefings and workshops at annual provider meetings
- Educate professional staffs
- 76 HAWCs lead

OPERATIONALIZE EFFICIENT RUNNING
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Beyond Running

AF READY TO SPRINT AHEAD
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Beyond Running

MILITARY PHYSICAL TRAINING SINGULARITY
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Summary
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Comparison of the 1.5 Mile Run Times at 7,200 Feet and Simulated 850 Feet in a Hyperoxic Room

HQ USAFA/ADPH

Lt Col Michael Zupan

The 1.5-mile run test was developed by Dr. Ken Cooper as an easy, inexpensive, and relatively accurate way to estimate VO2 max, or aerobic fitness levels, in large groups of AF personnel. In 2004 the AF fitness program began using the 1.5-mile run to estimate an airman's aerobic capacity. An altitude adjustment was implemented in 2005 for airmen stationed above 5,000 ft. In 2010, a new AF fitness test program was implemented; however, the 1.5-mile altitude adjustment for moderate altitude AF bases was removed. This study was conducted to investigate if a significant difference in aerobic performance exists between moderate altitude and sea level and, if it does exist, to what extent. The study was reviewed and approved by the USAFA IRB with all subjects signing an ICD. Fifty-five, 38 male and 17 female, subjects participated in the study. Subjects completed a VO2max test followed by two 1.5-mile runs, one at 7,200 ft, and one at simulated 850ft (~26% O2). During the runs, subjects only were aware of their test distance and could adjust the treadmill speed based on how they were feeling. Treadmill speed, elapsed test time, heart rate, and testing environment were unknown during all runs. Results were analyzed using an ANOVA. The average max VO2 was 48.6 mL.kg.-1min-1. A 30.6 seconds, or 4.2%, significant difference ($p<.001$) was observed between the two runs. These differences were mainly due to a decreased hemoglobin oxygen saturation ($p<.001$). Our recommendation is that an altitude adjustment for the AFT be reinstated.

HQ U.S. Air Force Academy

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Comparison of the 1.5 Mile Run Times at 7,200 Feet and Simulated 850 Feet in a Hyperoxic Room

Lt Col Michael Zupan, Ph.D.
Director, USAFA Human Performance Lab

Study Objective

This study investigated if there are differences in aerobic performance between altitude and simulated sea level environments and if differences are evident between conditions, to what extent?

Background Information

- **1968** - Dr. Ken Cooper develops the 12 minute run fitness test as an easy, inexpensive and relatively accurate way to estimate VO_2 max, or aerobic fitness, in large groups of Air Force personnel. (R = .897)
  - Based on results of 115 airmen
  - Better indicator of cardiovascular fitness than the 600 yard run.
- Later Dr. Cooper developed the 1.5 mile test
- **1992** - Cycle ergometry test was implemented to "predict" VO_2 max.

Background Information (cont)

- **2004** - New Air Force fitness program was implemented that once again used the 1.5 mile test.
- **2005** - An altitude adjustment was implemented for airmen stationed above 5,000 ft. (1.75 pts)
- **2010** - New Air Force fitness test program was implemented, which still used the 1.5 mile run to test aerobic fitness, but the altitude adjustment for the Air Force Bases located at moderate altitude is removed.

Background Information (cont)

"The high altitude calculation was removed as all individuals are already given a temporary exemption of six weeks to adapt to the altitude differences between locations"
and
"With six weeks to acclimatize and continue training at altitude, members' 1.5 mile run performance should not be appreciably degraded"
and
"Exercise research indicates that a score adjustment for people taking the revised Air Force Physical Fitness Test at higher altitudes is not needed. The VO_2 max or aerobic fitness, the factor we are measuring with the 1.5 mile run, is not measurably altered in a non-acclimated member testing from sea level up to 7,000 feet."

(Air Force Fitness Program Web Site FAQ)

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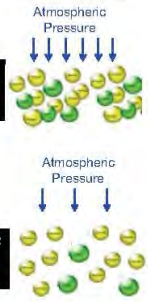
Background Information (cont)

- As altitude is increased, barometric pressure decreases
  - Results in less oxygen per given volume of air than at sea level
  - Known as hypobaric hypoxia
- Current research shows that total acclimatization can take up to 4-6+ months (Brothers, 2008; Brothers, 2007)
- Aerobic endurance still is impaired even with total acclimatization (Brothers, 2008; Brothers, 2007)
- Training intensities are reduced at altitude which results in deconditioning of the body (TB 505, 2010)
- To date, it is unknown the exact amount of decrement associated with various levels of the hypobaric hypoxic environments.



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Protocol

- Time requirement for each subject was ~2 hours
  - **30 min- ICD and VO_{2max} introduction**
  - **30 min- VO_{2max} and DXA scan**
  - **30 min- 1.5 mile run in first condition**
  - **30 min- 1.5 mile run in opposite condition**
- All 1.5 mile runs were performed in the Colorado Altitude Tent (CAT) in normal moderate altitude environment (~7,200 ft) or ***normobaric hyperoxic environment*** (oxygen content increased while barometric pressure stayed the same) to simulate ~850 ft.
- Order of running conditions were randomized.
- Only 24 to 72 hours between 1.5 mile runs
- Distance was the only factor known by the subjects during the 1.5 mile runs

Oxygen Content in CAT (~26.5%)

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Creating a Hyperoxic Environment

Partial Pressure of O_2

SL
.21 * 760 mmHg = PO_2 of **160 mmHg**

7,200 FT
.21 * 570 mmHg = PO_2 of 120 mmHg

CAT
.265 * 570 mmHg = PO_2 of **152 mmHg**

Oxygen content at Altitude (20.9%)

Oxygen content in CAT (~26.5%)

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Dual Energy X-Ray Absorptiometry (DXA) Scanner for Body Composition

- "Gold Standard" for body composition
- Assessments provide :
  - % fat mass
  - % lean body mass
  - Bone density

REGION	TOTAL	Left	Right
Arms	14.2%	14.3%	14.1%
Legs	23.1%	23.9%	23.1%
Trunk	25.3%	25.3%	25.3%
Total	22.3%	22.2%	22.40%

% Body Fat

VO_2 Max test

- All VO_2 max tests were performed at altitude
- Subjects were asked to continue running until they reached volitional fatigue
- Protocol

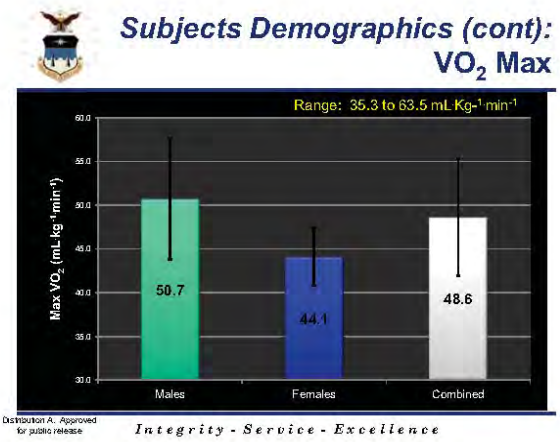
Test Time (min)	Stage Time (min)	Speed (mph)	Grade (%)	Position
0-1	1:00	0	0	Standing
2-3	2:00	2.0	0	Walking
4-5	2:00	7.0 m, 6.0 f	0	Running
6	1:00	7.0 m, 6.0 f	2	Running
7	1:00	7.0 m, 6.0 f	4	Running
8	1:00	7.0 m, 6.0 f	6	Running
9	1:00	7.0 m, 6.0 f	8	Running
10	1:00	7.0 m, 6.0 f	10	Running
11	1:00	7.0 m, 6.0 f	11	Running
12	1:00	7.0 m, 6.0 f	12	Running
13	1:00	7.0 m, 6.0 f	13	Running
14	1:00	7.0 m, 6.0 f	14	Running
End of Test	Until HR <120	2.0	0	Active Recovery

Max VO_2

Study Participants

- Fifty-five, non smoking, male and female subjects signed informed consent documents (ICD) and completed DXA and VO_{2max} tests
- All subjects had to be living continuously in Colorado Springs for at least 6 weeks.
- Three subjects did not complete the 1.5 miles run tests due to AF commitments and non-study related injuries
- *Subjects demographics:*

	n	DXA (%BF)	Age (yrs)	Weight (lbs)	Height (in)
Males	38	16.4 ± 7.6	32.3 ± 6.5	173 ± 24	71.7 ± 3.1
Females	17	24.9 ± 4.7	33.6 ± 6.9	132 ± 18	64.7 ± 2.2
Total	55	19.0 ± 7.9	32.7 ± 6.6	160 ± 29	69.5 ± 4.4


Subjects Demographics (cont):
VO2 Max
Range: 35.3 to 63.5 mL·Kg-1·min-1
Max VO2 (mL·kg-1·min-1)
50.7
44.1
48.6
Males
Females
Combined
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Results
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13/22


1.5 Mile Run times at ALT and SL
Alt
SL
Time (sec)
Distance (miles)
0.25
0.50
0.75
1.00
1.25
1.50
* p<0.001
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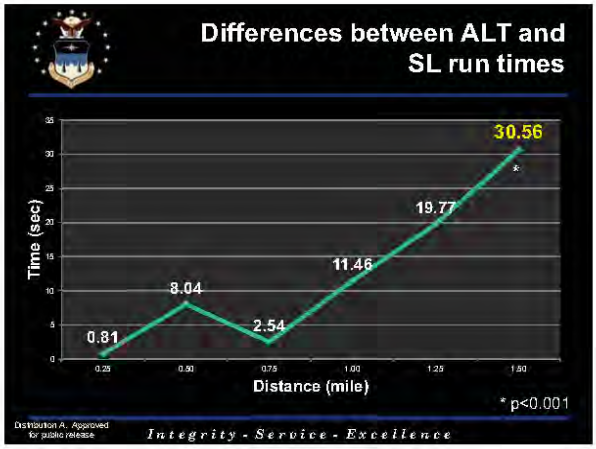
Differences between ALT and SL run times
Time (sec)
0.81
8.04
2.54
11.46
19.77
30.56
Distance (mile)
* p<0.001
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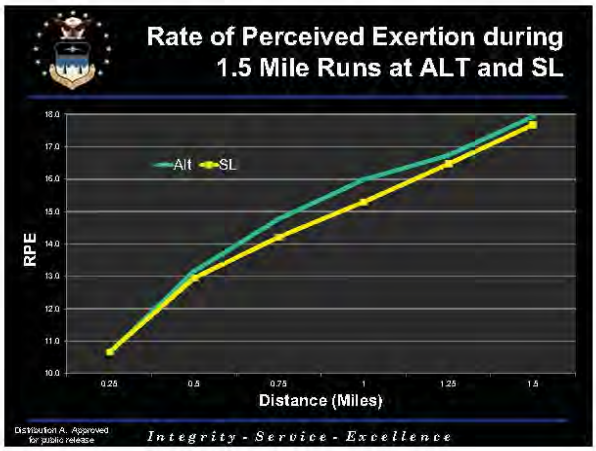
Rate of Perceived Exertion during 1.5 Mile Runs at ALT and SL
Alt
SL
RPE
Distance (Miles)
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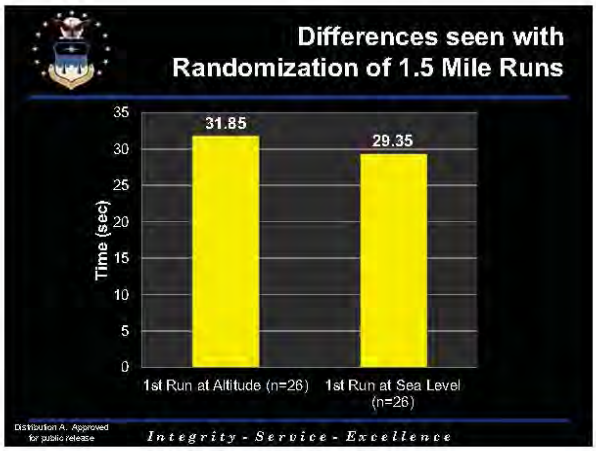
Differences seen with Randomization of 1.5 Mile Runs
31.85
29.35
Time (sec)
1st Run at Altitude (n=26)
1st Run at Sea Level (n=26)
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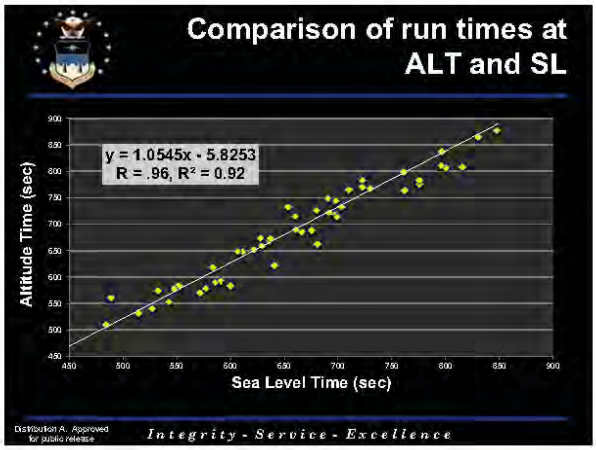
Comparison of run times at ALT and SL
y = 1.0545x - 5.8253
R = .96, R² = 0.92
Altitude Time (sec)
Sea Level Time (sec)
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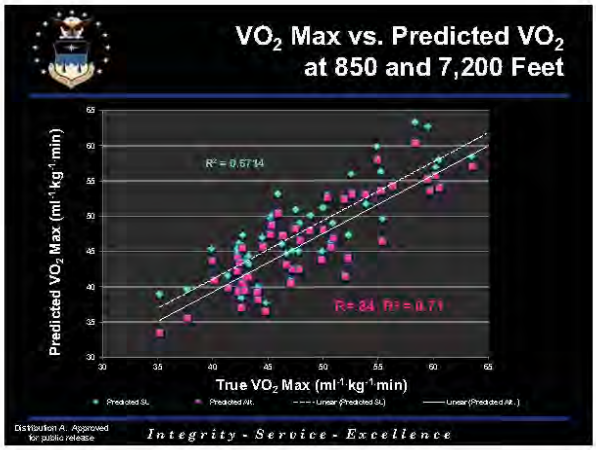
VO2 Max vs. Predicted VO2 at 850 and 7,200 Feet
R² = 0.6714
Predicted VO2 Max (ml·kg-1·min)
True VO2 Max (ml·kg-1·min)
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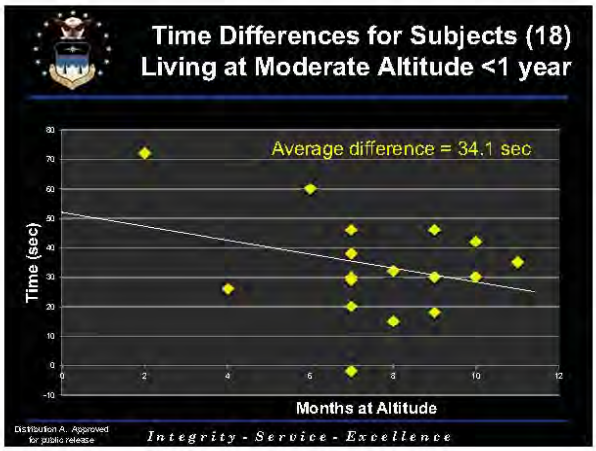
Time Differences for Subjects (18) Living at Moderate Altitude <1 year
Average difference = 34.1 sec
Time (sec)
Months at Altitude
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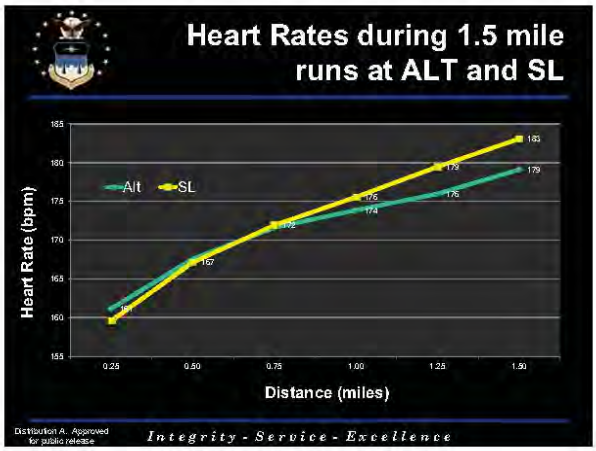
Heart Rates during 1.5 mile runs at ALT and SL
Alt
SL
Heart Rate (bpm)
Distance (miles)
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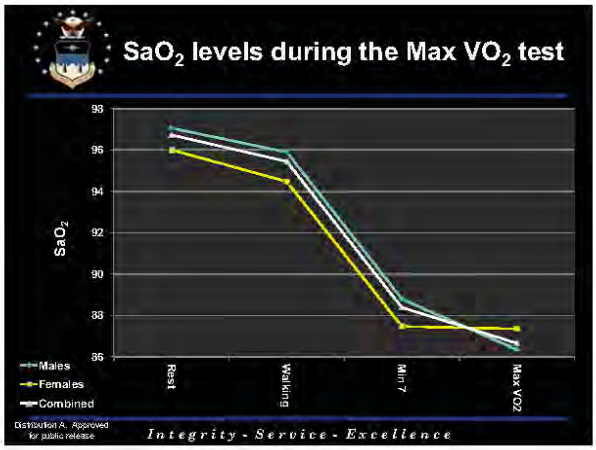
SaO2 levels during the Max VO2 test
SaO2
Males
Females
Combined
Rest
Walking
Min 7
Max VO2
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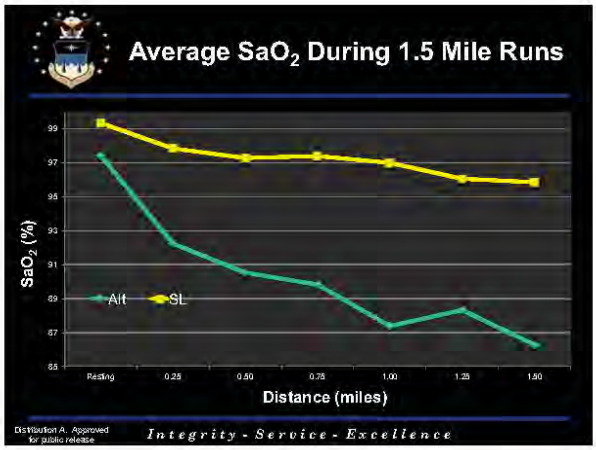
Average SaO2 During 1.5 Mile Runs
SaO2 (%)
Alt
SL
Resting
Distance (miles)
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Conclusions

- A 30.6 seconds, or 4.2% decrease in 1.5 mile running times was measured when running at ~850 ft compared to 7,200 ft.
- These differences were mainly due to a decreased hemoglobin oxygen saturation associated with running at altitude with lower O_2 partial pressures.
- HR and RPE were not significantly different between runs
- Our recommendation is that an altitude adjustment for the Air Force fitness test be reinstated for airmen testing at moderate altitude bases.

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Acknowledgments

I wish to acknowledge the help of the following individuals in data collection early analysis of the results.

Dustin R. Bakkie - Western State College
Jennifer A. Malagon - Colorado State University
Jessica A. Malagon - Colorado State University
Kristin Perdue - University of Northern Colorado

- **This work was supported by Air Force Medical Support Agency(AFMSA/SG9)**

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Can a 10-minute Warm-up Reduce Musculoskeletal Injury in Air Force Academy Cadets?

Uniformed Services University, Injury Prevention Research Lab

Dr. Sarah De La Motte

Musculoskeletal injury (MSK-I) is the leading cause of lost duty time and morbidity in the military. The short and long-term consequences from MSK-I can be career-threatening, if not career-ending, and decrease force readiness. New data show major risk factors for MSK-I in athletic populations can be easily identified and are readily modifiable through prevention programs targeting poor movement patterns. However, maximal MSK-I prevention program design & effectiveness in military environments have not been determined. We are working with the US Air Force Academy (USAFA) Department of Physical Education (DPE) to study the effects of a 10-minute neuromuscular warm-up program performed in required physical training sessions. Sections of a required freshman P.E. class will be randomized to perform a neuromuscular warm-up developed to address previously identified MSK-I risk factors, or a traditional warm-up program. Neuromuscular warm-up sessions will be professionally supervised, with cadets receiving real-time feedback on program performance, including technique & correction cues. Rates of lower extremity injury and biomechanical changes in movement pattern will be compared between groups. Post-training jump-landing assessment data will be compared with pre-training data to determine the neuromuscular warm-up program's effect on "high-risk" movement patterns and coupled with MSK-I incidence to determine program effectiveness. Pre and post-data will also be compared with subsequent testing sessions in a subsample of cadets to determine washout of training effect and optimum periodicity of warm-up training. This research will provide feasibility and injury incidence data for a larger definitive trial of MSK-I focused prevention programs in the Air Force.

Can a 10-minute Warm-up Reduce MSK-Injury in USAFA Cadets?

What We Know So Far

Sarah J. de la Motte, PhD, ATC
Anthony I. Beutler, LTC, MC, USAF
Injury Prevention Research Laboratory
Uniformed Services University

Objectives

- Why MSK-injuries are a big deal
- What is JUMP-ACL?
- What's going on now at USAFA
- The way forward

Injuries are a Major Problem in the Military!

- Non-combat Musculoskeletal (MSK) Injuries in the Military:
  - 1.6 million medical encounters/yr
  - **#1 cause** of lost duty days
  - Biggest health problem of the military services

Jones BH, et al: Medical Surveillance of Injuries in the U.S. Military: Descriptive Epidemiology and Recommendations for Improvement. American Journal of Preventive Medicine 2010;38(1S):S42-S60.

MSK Injuries are a Major Problem during Deployments!

- 34% of deploying troops sustained a non-combat MSK injury
- The most common reasons for medical air evacuation:
  - Non-combat MSK injuries (24%)
  - Combat injuries (14%)

Cohen SP et al: Diagnoses and factors associated with medical evacuation and return to duty for service members participating in Operation Iraqi Freedom or Operation Enduring Freedom: a prospective cohort study. Lancet 2010;375:301-309.

Consequences of MSK Injury during Deployment

Consequences
- Force Depletion
- Decreased Readiness

Long-Term Consequences
- Loss of Camaraderie
- Loss of Unit Cohesion
- Mission Compromise

Consequences of MSK Injury during Training

Short-Term Consequences
- Injury during Basic Train
  - 25% proceed to "F Discharge" (m

Long-Term ...quences
- Tr... njury = ↑ Arthritis Risk
  - ... ACL Injury:
    - No Surgery: **>80%** OA Risk in 15 ys
    - "Good" Surgery: **>80%** OA Risk in 15 ys

- Knapik, Med Sci Sports Ex, 2001

•PRIMARY PREVENTION IS KEY!!

Previous Injury Prevention Efforts

- **Limited Success**:
  - Cost
  - Modifiable vs. Non-Modifiable Risk Factors

Not-Readily Modifiable:
- Female Gender
- BMI
- Fitness Level
- Smoking

Previous Injury Pr...n Efforts

- **Limited Success**
  - Cost
  - Modifi... Ris...

...eadily Modifiable:
- Strength
- Equipment
- Training Schedule
- ✓ **Movement Patterns**

•BUT WHICH MOVEMENT PATTERNS??

Objectives

- Why MSK-injuries are a big deal
- What is JUMP-ACL?
- What's going on now at USAFA
- The way forward

JUMP ACL
MONITORING AND PREVENTING ACL INJURIES

JUMP-ACL:
Joint Undertaking to Monitor and Prevent ACL Injury

A Prospective Cohort Study of Modifiable Risk Factors for ACL Injury

An "Almost Final" Report

Collaborators

- Anthony Beutler, MD, MC, USAF
  - Uniformed Services University
- Stephen W. Marshall, PhD
  - University of North Carolina, Chapel Hill
- Darin Padua, PhD, ATC
  - University of North Carolina, Chapel Hill
- William E. Garrett, MD, PhD
  - Duke University

JUMP ACL
MONITORING AND PREVENTING ACL INJURIES

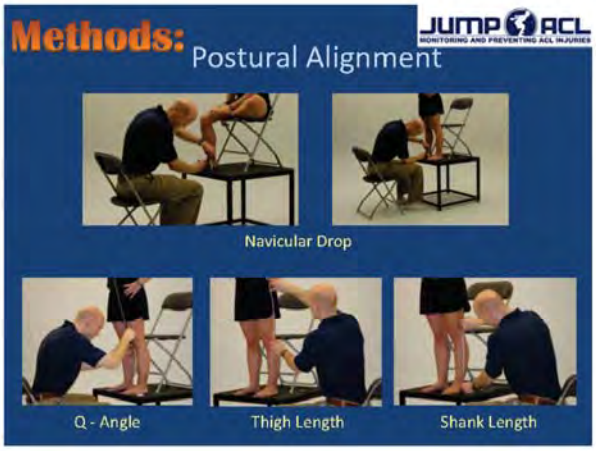
What modifiable risk factors predict ACL injury risk?

- 5 year trial at 3 military academies
- 500/academy/year
  - ~40% female
  - ~ 6,000 subjects
  - 15,000 man-years
- Goal = Capture 100 Primary ACL injuries


ACL Injury in Military Academies
Air Force, Army, Naval Academies
• 13,500 cadets x 3.3% ACL injury rate
>110 ACL Injuries/year
• ACL Injury: 9 mo's lost/limited time
90 man-years lost annually
• Academy education = $100K/yr
$9 million/yr in lost/limited training time


Methods:
JUMP ACL
Informed Consent &
Baseline Questionnaire


Methods:
JUMP ACL
Strength Testing
Hip Extension
Hip Abduction
Hip External Rotation
Hip Internal Rotation
Knee Flexion
Knee Extension


Methods:
JUMP ACL
Postural Alignment
Navicular Drop
Q - Angle
Thigh Length
Shank Length

Methods:

JUMP ACL MONITORING AND PREVENTING ACL INJURIES

Jump-Landing Biomechanics

- Drop height = 30 cm
- Horizontal distance = 50% body height
- Jump for maximum vertical height after landing
- Collected 3-D joint kinematics & kinetics
  - Electromagnetic system (1440 Hz) & force-plate (144 Hz)

Results: "Almost Final"

Non-Contact / Indirect Contact ACL Injuries

	No		Yes	
	n	Pct	n	Pct
Females	2,395	39%	39	40%
Males	3,631	61%	59	60%
Total	6,026	100%	98	100%

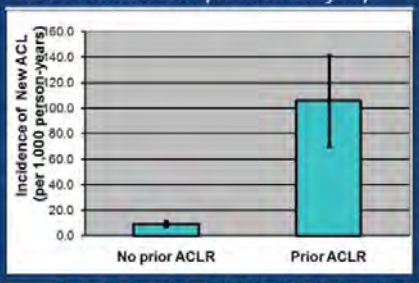
Results

Baseline Questionnaire

JUMP ACL MONITORING AND PREVENTING ACL INJURIES

JUMP ACL MONITORING AND PREVENTING ACL INJURIES

Previous History of ACL Injury



Rate Ratio=6.4; 95%CI: 4.9, 15.7; P<0.01

Results

JUMP ACL
MONITORING AND PREVENTING ACL INJURIES

Jump-Landing Biomechanics

Human Movement Risk Factors for Subsequent ACL Injury: "Lab" Findings

- Knee in Valgus at Initial Ground Contact
  - RR 2.0 for non-contact ACL
- Rapid Hip Internal Rotation on Contact
  - RR 6.8 for non-contact ACL

Table 3. Biomechanical Risk Factors for ACL Injury

	All ACL Injuries		Non-contact	
Risk Factor	Rate Ratio[1] (95%CI)	Wald p-Value	Rate Ratio (95%CI)	Wald p-Value
Valgus Knee Angle at Initial Ground Contact	1.9 (1.0, 3.7)	0.053	2.0 (0.7, 5.6)	0.195
Hip Rotation >16deg/sec over the Absorption Phase	2.5 (1.2, 5.0)	0.010	6.8 (1.8, 25.3)	0.004
Average Vertical Ground Reaction Force > 150% of Body Weight in Post-Impact Phase	2.8 (1.3, 6.2)	0.009	4.3 (1.3, 14.0)	0.017

[1]Adjusted for all other variables in the table in multivariate Poisson models; unadjusted estimates are similar

Key Points

- Risk Factors ≠ Injury Mechanisms

•But we can't get everyone to the lab!

So…

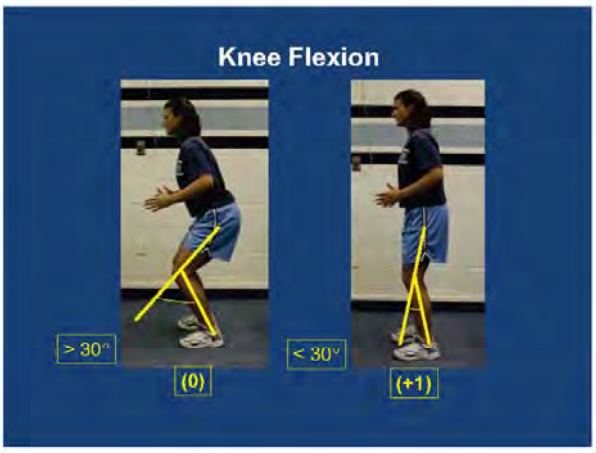
- ↑ Knee valgus, ↑ Hip IR, ↑ Knee Varus force, Hard/inefficient landing

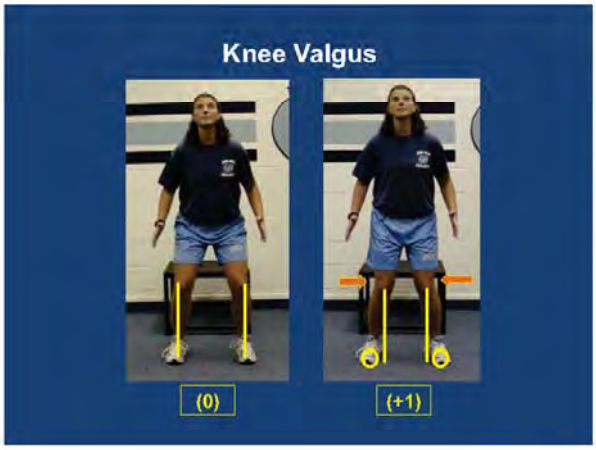
Landing Error Scoring System (LESS) Clinical Movement Analysis Tool

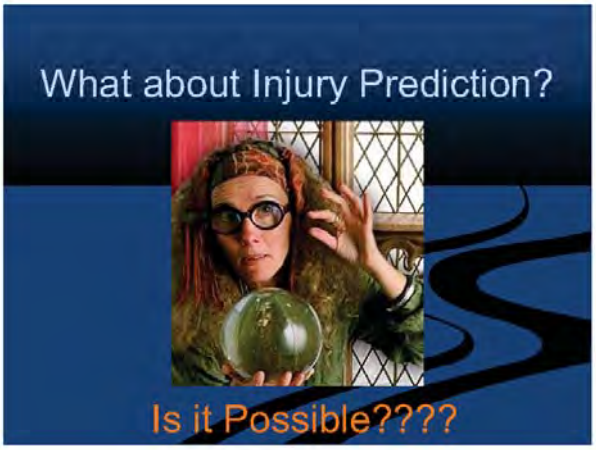
Sagittal View

Frontal View

1. Knee Flexion @ Initial Contact: > 30 degrees
   Yes (0)
   No (+1)
2. Knee Valgus @ Initial Contact: Knees over midfoot
   Yes (0)
   No (+1)
3. Hip Flexion @ Initial Contact: Hips are flexed
   Yes (0)
   No (+1)
4. Trunk Flexion @ Initial Contact: Trunk is flexed
   Yes (0)
   No (+1)
5. Lateral Trunk Flexion @ Initial Contact: Trunk is vertical
   Sternum centered over hips (0)
   Lateral deviation of sternum over hips (+1)
6. Ankle Plantar Flexion @ Initial Contact: Toe to heel
   Yes (0)
   No (+1)
7. Foot Position @ Initial Contact: Toes > 30 of ER
   Yes (+1)
   No (0)
8. Foot Position @ Initial Contact: Toes > 30 of IR
   Yes (+1)
   No (0)
9. Stance Width @ Initial Contact: < Shoulder width
   Yes (+1)
   No (0)
10. Stance Width @ Initial Contact: > Shoulder width
    Yes (+1)
    No (0)
11. Initial Foot Contact: Symmetric
    Yes (+0)
    No (+1)
12. Knee Flexion Displacement: > 45 degrees
    Yes (0)
    No (+1)
13. Knee Valgus Displacement: ≥ great toe
    Yes (+1)
    No (0)
14. Hip Flexion Displacement: Hips flex more than at initial contact
    Yes (0)
    No (1)
15. Trunk Flexion Displacement: Trunk flexes more than at initial contact
    Yes (0)
    No (1)
16. Joint Displacement (Sagittal Plane)
    Soft (0)
    Average (+1)
    Stiff (+2)
17. Overall Impression
    Excellent (0)
    Average (+1)
    Poor (+2)







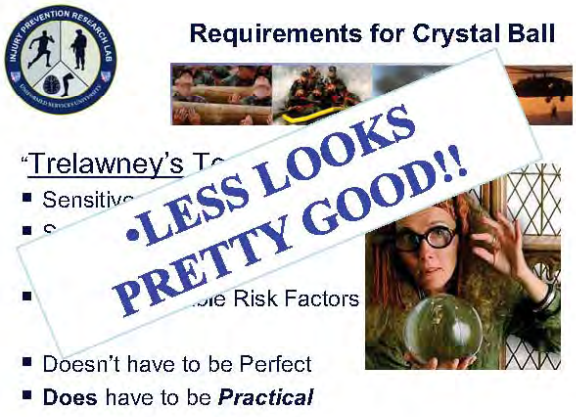
Requirements for a Crystal Ball

"Trelawney's Tests"

- Sensitive
- Specific
- Identify Modifiable Risk Factors
- Doesn't have to be Perfect
- **Does** have to be ***Practical***

Predictive Validity

- Preliminary data suggests **LESS** may be a valid **clinical assessment** of **ACL injury risk**
  - ↑ LESS scores in ACL-injured youth soccer players
  - **Sensitivity = 83%**
  - **Specificity = 67%**
- Similar to **laboratory** external knee valgus
  (Hewett et al, 2005)
  - Sensitivity = 78%
  - Specificity = 67%

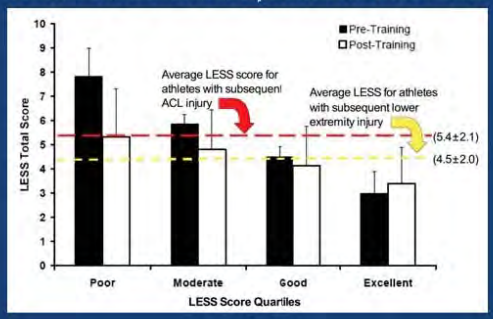


Dynamic Movement Enhancement Program

- Developed from *prospective* JUMP-ACL data
- 10 minute warm-up
- 2-3 times/week

In Youth soccer:
- 70% ACL Reduction!
- 50% decrease in **ALL** Lower Extremity injuries!

What does the crystal ball tell us?



Existing Injury Prevention Programs

DIME stacks up pretty well!

- PEP - Mandelbaum:
  - prevents 70% non-cont ACL in female soccer
  - 30 minutes, 3-5 times/week
- Cincinnati Sports - Hewett:
  - Lower incidence of knee injuries
  - 60-90 minutes, 4-5 times/week
- Handball/Floorball - Olsen & Pasanen
  - 50-65% reduction in ALL lower extremity injuries
  - 20-30 minutes, 3-4 times/week

Objectives

- Why MSK-injuries are a big deal
- What is JUMP-ACL?
- **What's going on now at USAFA**
- The way forward

Next Step: NOW at USAFA

JUMP-ACL2

•Implement *Proven* Injury Prevention Program

- Use DIME to determine its effectiveness in Academy
- Pre & Post intervention assessment
- Approximately 50% of cadets get DIME in freshmen PE
  - Other half continue with standard USAFA warm-up
- Tie movement pattern changes to MSK-I outcomes

Initial Screen

- ALL incoming cadets in 2011 screened using the LESS (N~1200)

Exercise Intervention - DIME

- Exercises incorporated into required freshman PE class as a regular warm-up
  - 50% of cadets randomized to receive usual warm-up
  - 50% of cadets receive **DIME program** under **professional supervision** by **trained movement specialist**
    - Changes in movement pattern require coaching, reinforcement & active feedback
    - Thank you, DSOC!

Post-Assessment

- Injury Risk Screen repeated after completion of PE class
  - How did movement patterns/LESS score improve?
- Sub-sample screened at regular intervals to assess decay
  - How long do these changes last?
- ACL & lower extremity injury data obtained for next 12 mo
  - USAFA Cadet Injury Tracking System coming online Fall 2011
  - The Holy Grail!

Objectives

- Why MSK-injuries are a big deal
- What is JUMP-ACL?
- What's going on now at USAFA
- The way forward

JUMP-ACL2

USAFA vs. USMA

10 Min Injury Prevention, Movement Re-Training Program

- Changes in movement pattern require coaching, reinforcement, and active feedback

Summer Basic Cadet Training Versus Freshman PE Class

- **USAFA – Freshman PE**; USMA – Summer BCT

Capture Movement Pattern Chances & Injury Outcomes

- Preliminary Results
  - Movement Pattern Changes Hard to Capture
  - 5X ↓ LE injuries in Intense Supervised Program

Goals for the Future

- **Prevent** Anterior Cruciate Ligament (ACL) & lower-extremity injuries in Academy cadets
- **Determine** proper supervision method for exercise instruction (professional vs cadet led)
- **Evaluate** for decay of movement pattern change and training effect
- **Using this knowledge, create** a proven, portable, user-friendly program to translate into Big Military

Objectives - Recap

- Why MSK-injuries are a big deal
- What is JUMP-ACL?
- What's going on now at USAFA
- The way forward

Acknowledgements

- LTC Anthony Beutler
- USAFA Department of Physical Education
- Defense Safety Oversight Council

Questions?

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sarah.delamotte@usuhs.mil 301-295-9460

Anti-retinal Antibodies as Biomarkers for Laser Induced Retinal Injuries in Rabbits

Summa Health System

Dr. Rachida Bouhenni

PURPOSE: Retinal injuries affecting the photoreceptors and/or the retinal pigment epithelium (RPE) may result in leakage of retinal-specific proteins into the systemic circulation. These proteins could be detected in body fluids following the injury and vary with the severity of the injury and during the subsequent recovery period.

METHODS: Using a continuous 532 nm laser, 50 spots of mild (MVL), moderate (GII), or severe (GIII) laser lesions were created in retinas of Dutch Belted rabbits (n=12/grade). Serum and saliva were collected from treated and control animals at 1hrs, 4hrs and 24hrs following laser treatment. Retinal-specific proteins were detected using Liquid Chromatography/Tandem Mass spectrometry. Statistical analyses were performed using One way ANOVA. $P<0.05$ was considered significant.

RESULTS: Retinal-specific proteins were detected in both saliva and serum samples at all time points after laser injury. Most proteins were detected in the samples treated with MVL at 4hrs, followed by GII and GIII laser lesions. Some of the proteins were common to more that one laser grade. Although, more proteins were detected following treatment with mild lesions, and at 4 hrs after treatment, the differences between groups were not significant. CONCLUSION: Retinal-specific proteins were detected in both saliva and serum of rabbits following laser treatment. The numbers of proteins detected did not vary with severity and time following injury. The biomarker response appears transient, peaks at 4 hours after laser treatment and is reduced at 24hrs.These proteins could be used as biomarkers for laser induced retinal injuries in military operations.

Anti-retinal Antibodies as Biomarkers for Laser Induced Retinal Injuries in Rabbits

Rachida Bouhenni, PhD
Summa Health System, Akron, OH

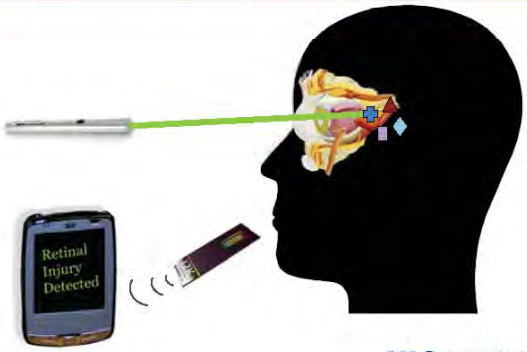
Background & Significance

- Laser sources can cause ocular trauma/retinal damage
  - Laser weapons
  - Laser sights
  - Some remote sensing instruments
  - Handheld laser pointers
- War fighters and other operators are at increased risk
- Some lesions are asymptomatic and almost impossible to detect in routine examinations
- Non-invasive diagnostic tests to detect molecular signatures of retinal injuries are needed.

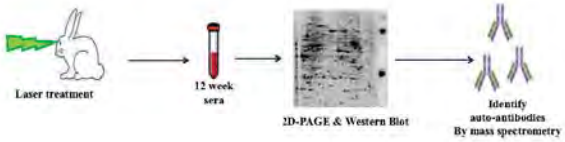
Hypothesis

- Laser causes photoreceptor and RPE cell death and violates the Blood Retina Barrier
- Disruption of the Blood Retinal Barrier following laser exposure leads to the release of retinal proteins into the blood circulation.
- These proteins may initiate an immune response, resulting in auto-antibodies that are detectable in the serum 12 weeks later.
- These auto-antibodies could serve as molecular biomarkers for retinal injuries caused by laser.

Vision for Clinical Application



Approach



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Experimental Design Overview

- **Experiment 1 (n=72 rabbits):**
  - Variable: Laser injury grades
    - Different laser grades (MVL, GII, GIII)
    - Fixed lesion number (50 lesions, 1 eye)
- **Experiment 2 (n=72 rabbits)**
  - Variable: Exposure levels
    - Different injury profiles (5, 10, 50 lesions)
    - Fixed laser grade (MVL)
- **Experiment 3 (n= 46 rabbits)**
  - Variable: # of laser exposures
    - 2 or 3 MVL laser treatments, 50 lesions per treatment
    - 1 month between treatments

Blood collected at 12 weeks after exposure

The animal protocol was approved by USAF animal research program and the IACUC committee at NEOMU

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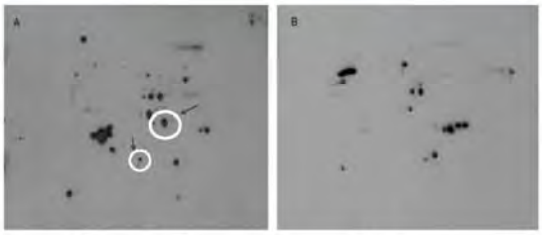
2D Western Blot



Laser treated (GII) Mock

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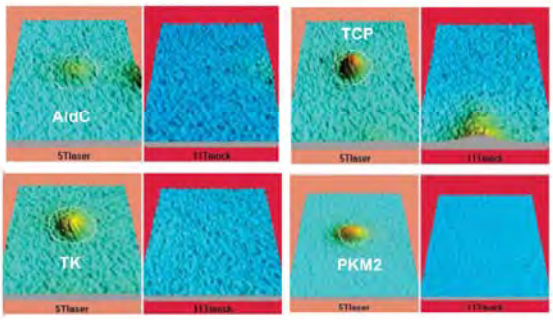
Spot Analysis and Selection



Only spots present in 2 or more laser and none of the controls are selected

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Spot Excision

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Experiment 1 Results

# animals	Protein description	Laser Grade
2 of 2	Fructose bisphosphate aldolase C	MVL
4 of 4	Dihydropyrimidinase related protein 2	GII
3 of 4	Triosephosphate isomerase	GII
2 of 4	Fructose bisphosphate aldolase C	GII
2 of 4	Transketolase	GII
2 of 4	Transitional endoplasmic reticulum ATPase	GII
2 of 4	Serotransferrin	GII
2 of 4	Cofilin-1	GII
2 of 4	Alpha enolase	GII
2 of 4	T-complex protein 1 subunit zeta	GII
2 of 4	Pyruvate kinase isozymes M1/M2	GII
2 of 4	Elongation factor 1-alpha 1	GII
3 of 3	Probable ATP-dependent RNA helicase DDX17	GIII

Auto-antigens are confirmed by size and IP

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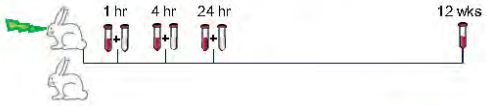
Experiment 2 Design Details

- 4 Groups (n=72)
  - All MVL Injuries
    - Group 1 = 5 lesions (n=18)
    - Group 2 = 10 lesions (n=18)
    - Group 3 = 50 lesions (n=18)
    - Group 4 = Mock Control (n=18)



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Experiment 2 results

- No auto-antibodies were detected in the 5 or 10 lesions treated animals
- The 50 lesions treated animal's samples in process
- Repeated to reproduce results

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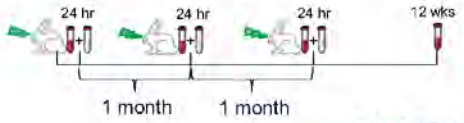
Experiment 3 Design Details

- 5 Groups (n=46)
  - All MVL lesions, 1 month intervals
    - Group 1 & 3 =1 laser exposure, 50 lesions
    - Group 2 & 4 =2 laser exposures, 100 total lesions
    - Group5 = 3 laser exposures, 150 total lesions



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Experiment 3 results

# animals	Protein description	/# of laser treatments
3 of 3	Glutamine synthetase	1
3 of 3	**Pyruvate kinase isozymes M1/M2**	**1**
2 of 3	Ubiquilin-1	1
2 of 3	**T-complex protein 1 subunit zeta**	**1**
2 of 3	**Dihydropyrimidinase related protein 2**	**1**
2 of 3	Tubulin beta-2 chain	1
2 of 3	Bifunctional purine biosynthesis protein	2
2 of 3	Aspartate aminotransferase	2
2 of 2	Heme-binding protein 2	3
2 of 2	Beta enolase/Alpha enolase	3
2 of 2	Tubulin alpha-1 chain/Tubulin beta-2 chain	3

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Top Candidate auto-antibodies

UniProt Accession	Protein description	MVL	GII	GIII
O02675	Dihydropyrimidinase-related protein 2	2/8	4/4	0/3
Q9GKW3	Fructose-bisphosphate aldolase C	5/8	2/4	0/3
O77622	T-complex protein 1 subunit zeta	2/8	2/4	0/3
P11974	Pyruvate kinase isozymes M1/M2	6/8	2/4	0/3

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Other candidates

UniProt Accession	Protein description	MVL	GII	GIII
P00939	Triosephosphate isomerase	1/8	3/4	0/3
Q6B855	Transketolase	0/8	2/4	0/3
P03974	Transitional endoplasmic reticulum ATPase	0/8	2/4	0/3
P19134	Serotransferrin	0/8	2/4	0/3
Q5E9F7	Cofilin-1	0/8	2/4	0/3
Q9XSJ4	Alpha-enolase	0/8	2/4	0/3
P68105	Elongation factor 1-alpha 1	0/8	2/4	0/3
P15103	Glutamine synthetase	3/8	0/4	0/3
Q9UMX0	Ubiquilin-1	2/8	0/4	0/3
P69895	Tubulin beta-2 chain	2/8	0/4	0/3

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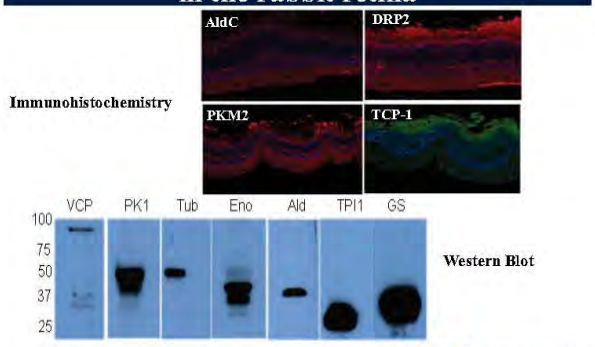
Expression of the candidate auto-antibodies in the rabbit retina



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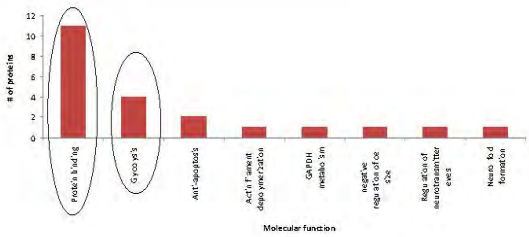
Molecular Function



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Conclusions

- Most auto-antibodies were detected in response to treatment with GII laser followed by MVL
- # of laser treatments resulted in different auto-antibodies.
- GIII laser may have caused protein degradation at the site of injury
- Most auto-antibodies were raised against proteins that have a function in glucose metabolism and protein binding
  (unregulated following treatment or abundant)
- that this approach may permit future development of new diagnostic methods for retinal injuries.
- A panel of 4 biomarkers may be used for detection of retinal laser injury: **DRP2, TPI, PKM and AldC**
- This approach may permit future development of new rapid diagnostic methods for retinal injuries

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Acknowledgments

This project is being developed under Contract Number FA7014-07-C-0047, with the U.S. Air Force Surgeon General's Office (AF/SG) and administered by the Air Force District of Washington (AFDW). The Air Force has not yet accepted the products depicted and issuance of a contract does not constitute a Federal endorsement of the University of Illinois at Chicago.

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Questions?

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Detection of Retinal Proteins in Saliva and Serum in Laser Induced Retinal Injuries in Rabbits

Summa Health System

Dr. Rachida Bouhenni

PURPOSE: Retinal injuries that affect the photoreceptors and/or the retinal pigment epithelium (RPE) may result in the leakage of retinal-specific proteins into the systemic circulation. This study was designed to determine whether an immune response is elicited after an acute retinal injury resulting in circulating anti-retinal antibodies in the serum.

METHODS: Fifty laser burns of different grades (minimally visible lesion (MVL), grade II (GII), or grade III (GIII) lesions) were created in the retinas of Dutch Belted rabbits. The degree of laser burns was confirmed by fundus imaging and histology. Serum samples were collected from the animals three months after the retinal injury. Candidate autoantigens were identified by two-dimensional western blots of rabbit retinal lysate probed with sera from either control or laser-treated animals. Candidate autoantigens were further characterized by immunohistochemistry to confirm their retinal localization. RESULTS: Seven and eleven protein spots were selected from the MVL and grade II laser-treated samples, respectively, for autoantigen identification. No protein spots were detected in the grade III laser-treated samples. Four candidate autoantigens were common to both MVL and GII lesions: Dihydropyrimidinase-related protein-2, fructose-bisphosphate aldolase C, chaperonin-containing T-complex polypeptide 1 subunit zeta, and pyruvate kinase isozyme. CONCLUSION: Induced retinal laser injuries resulted in circulating anti-retinal antibodies that were detectable three months after the injury. The response appeared to vary with the severity of the laser retinal damage. The identification of the candidate antigens in this study suggest that this approach may permit future development of new diagnostic methods for acute retinal injuries.

Detection of Retinal Proteins in Saliva and Serum Following Laser Induced Retinal Injuries in Rabbits

Rachida Bouhenni, PhD
Summa Health System, Akron, OH

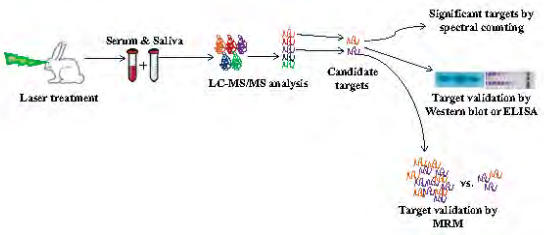
Background & Significance

- Laser sources can cause ocular trauma/retinal damage
  - Laser weapons
  - Laser sights
  - Some remote sensing instruments
  - Handheld laser pointers
- War fighters and other operators are at increased risk
- Some lesions are asymptomatic and almost impossible to detect in routine examinations
- Non-invasive diagnostic techniques to detect molecular signatures of retinal injuries are needed.

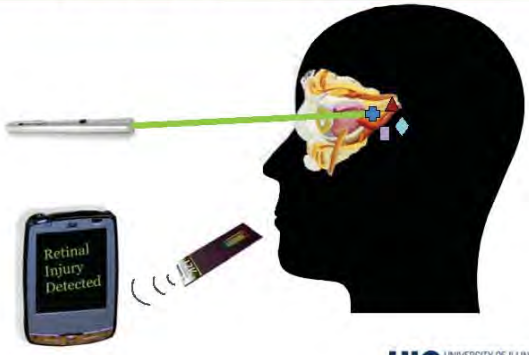
Hypothesis

- Laser causes photoreceptor and RPE cell death and violates the Blood Retina Barrier
- Protein from damaged cells can leak through the damaged blood retina barrier into the systemic circulation
- These proteins can be detected by proteomics in body fluids such as serum and saliva
- These proteins can be used as biomarkers for detection of laser induced retinal injuries.

Approach



Vision for Clinical Application



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Experimental Design Overview

- **Experiment 1 (n=72 rabbits):**
  - Variable: Laser injury grades
    - Different laser grades (MVL, GII, GIII)
    - Fixed lesion number (50 lesions, 1 eye)
- **Experiment 2 (n=72 rabbits)**
  - Variable: Exposure levels
    - Different injury profiles (5, 10, 50 lesions)
    - Fixed laser grade (MVL)
- **Experiment 3 (n= 46 rabbits)**
  - Variable: Time between exposures
    - 2 or 3 MVL laser injuries, 50 lesions per interval
    - 1 month between exposures

Saliva and serum samples were collected at 1hr, 4hrs and 24hrs.

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Laser Treatment Overview

- Frequency-doubled Nd:YAG laser (532 nm) mounted on a slit lamp.
  - MVL (Minimally Visible Lesion): 100 mw, 100 ms, 500 µm lesion
  - GII (Grade 2): 150 mw, 200 ms, 500 µm lesion
  - GIII (Grade 3): 300 mw, 200 ms, 500 µm lesion
- Mock control rabbits received anesthesia and pupil dilation but not laser.

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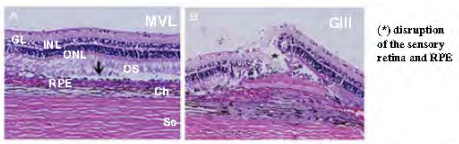
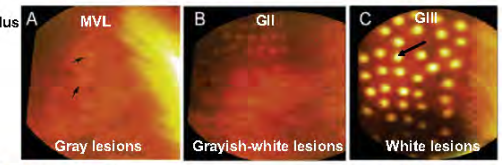
Confirmation of Laser Burns

Histology



(*) disruption of the sensory retina and RPE

Fundus



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LC-MS/MS

- Serum samples were fractionated by isoelectric focusing from pH 3-10 using a microrotofor (BioRad). Ten fractions were collected.
- 100 µl of either saliva or fractionated serum was polymerized into 15% acrylamide gel pieces.
- Gel pieces were incubated overnight in trypsin solution and digested proteins were extracted twice and allowed to dry.
- Dried samples were resuspended, sonicated, and extracted using a C18 ZipTip column (Millipore).
- Automated nano-flow HPLC-tandem mass spectrometry (LC-MS/MS) was performed.
- Eluted ions were electrosprayed at 1.75 kV.
- Data collected was blasted against the Uniprot mammalian database.

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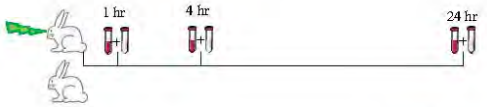
Experiment 1 Design Details

- 4 Groups (n=72)
  - MVL (n=18)
  - GII (n=18)
  - GIII (n=18)
  - Mock Control (n=18)



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Experiment 1 Results

Proteins detected in *saliva*	Frequency/total	Laser grade	Time point
CACNA1F	4/12	MVL (2), GII (1), GIII (1)	1hr, 4hrs, 24 hrs
CNG3 (CNGA3/CNGB3)	2/12	MVL, GII,	4hrs, 24hrs
PDE6 (A,B)	2/12	MVL, GII	1hr, 4hrs
CaBP1	1/12	MVL	4hrs

Proteins detected in *serum*	Frequency/total	Laser grade	Time point
CNG3(CNGA3/CNGB3)	3	MVL (1), GIII (2)	4hrs, 24hrs
PDE6 (B,C)	2	MVL (1), GII (1)	4hrs, 24hrs
Retinal oxidase	2	MVL(1), GIII (1)	24hrs
ABC4A	1	MVL	4hrs
RGS9	1	MVL	24hrs
Phosducin	1	GIII	4hrs

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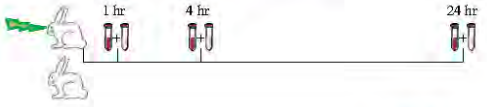
Experiment 2 Design Details

- 4 Groups (n=72)
  - All MVL Injuries
    - Group 1 = 5 lesions (n=18)
    - Group 2 = 10 lesions (n=18)
    - Group 3 = 50 lesions (n=18)
    - Group 4 = Mock Control (n=18)



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Experiment 2 Results

Proteins detected in *serum*	positive /10 pools*	Time point	# laser spots
Zinc finger protein	1	24hrs	5
Pleckstrin homology domain-containing family B member 1 (Pleckstrin homology	1	24hrs	50

- *No proteins were detected in saliva in Exp 2*
- *No definitive conclusions were made from this experiment*
- *The spot # does not affect the biomarker response*
- *Experiment was repeated, analysis in process*

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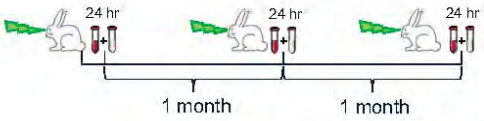
Experiment 3 Design Details

- 5 Groups (n=46)
  - All MVL lesions
    - Group 1 & 3 =1 laser exposure, 50 lesions
    - Group 2 & 4 =2 laser exposures, 100 total lesions
    - Group 5 = 3 laser exposures, 150 total lesions



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Experiment 3 Results

Proteins detected in *serum*	Frequency/total	Time point	# of laser treatments
CNGB3	1	24hrs	1
RPE65	1	24hrs	2
ABC4A	1	24hrs	2
Opsin 5	1	24hrs	1
Neural retina leucine (NRL) zipper protein	1	24hrs	1

- *No Proteins were detected in saliva*
- *No proteins were detected in the 3 laser treatment*
- *CNGB3 and PDE6 was detected again.*

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Candidate Biomarkers Detected in Serum

Retinal protein	MVL	GII	GIII
CNG3(CNGA3/CNGB3)	1 (4hrs)	-	2 (4hrs, 24hrs)
PDE6 (B,C)	1 (4hrs)	1(24hrs)	-
ABC4A	1 (4hrs)	-	-
RGS9	1(24hrs)	-	-
Phosducin	-	-	1(4hrs)
Retinal oxidase	1 (24hrs)	-	1 (24hrs)

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Candidate Biomarkers Detected in Saliva

Retinal protein	MVL (3 animals)	GII (3 animals)	GIII (3 animals)
CACNA1F	2 (1hr, 4hrs)	1 (4hrs)	1(24hrs)
CNG3 (CNGA3/CNGB3)	1(4hrs)	1(24hrs)	0
PDE6 (A,B)	1 (1hr)	1(4hrs)	0
CaBP1	1(4hrs)	0	0

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Biomarker Detection Over Time

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Conclusion

- Most biomarkers are detected in MVL at 1hr and 4hrs time point (transient).
- GIII results in a poor response, most likely because cells are dead and proteins are degraded.
- Number of laser lesions did not effect the biomarker response (experiment repeated).
- Intermittent laser treatments resulted in a different biomarker response
- A panel of 5 proteins can be used for detection of retinal laser injuries by LC/MS-MS (**CNGA3, CNGB3, PDE6A, PDE6B, PDE6C**)
- This approach may permit future development of new diagnostic methods for retinal injuries

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Future plans

- Validation of the candidate biomarkers using Western blot, ELISA or MRM.

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Acknowledgments

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Question?

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Serum Biomarker Responses in a Non-Human Primate Model of Acute Retinal Laser Injury

Summa Health System

Mr. Jeffrey Dunmire

PURPOSE: To identify unique proteomic signatures in sera indicative of retinal injury. METHODS: We used laser photocoagulation as a model of retinal injury in Rhesus macaques. Serum was collected from each animal at 4h, 1d, 3d, and 1w following a mock procedure and again following retinal laser treatment that produced either Grade 2 (moderately severe; GII, n=6) or minimally visible lesions (mild; MVL, n=6). Samples were analyzed by mass spectrometry and relative protein abundances were determined by spectral counting. Stringent filtering criteria and analysis by G-test, followed by Holm-Sidak correction for multiple comparisons, were used to determine statistical significance. Proteins with $p<0.05$ were considered significant. RESULTS: A total of 19 and 17 proteins were identified as significantly more abundant in sera following MVL and GII injury respectively. None of these proteins were common to both MVL and GII. However, among the 36 proteins, irrespective of injury severity, most were ontologically similar. Although most differences were unique to one time point, 4 proteins (CK18, PGK1, FUT3, and EPHA2) from MVL and 1 protein (DDX17) from GII showed differences at multiple time points after injury. For these proteins, maximal protein elevation between 4h and 3d was followed by a decrease to basal levels within 1w.

CONCLUSIONS: A serum biomarker response to both GII and MVL retinal injury was demonstrated. The proteomic signature was unique for each grade of injury and appeared transiently between 1-3d. Increased abundance of these proteins in serum may be useful markers for detection of acute retinal injury.

Serum Biomarker Responses in a Non-Human Primate Model of Acute Retinal Laser Injury

Jeffrey Dunmire
Ophthalmology Research
Summa Health System, Akron, OH

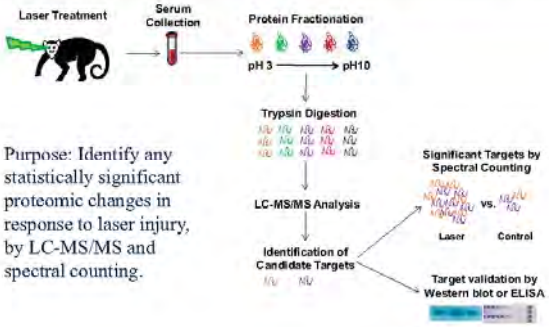
Background & Significance

- Laser sources can cause ocular trauma/retinal damage
  - Laser weapons
  - Laser sights
  - Some remote sensing instruments
  - Handheld laser pointers
- War fighters and other operators are at increased risk
- Some lesions are asymptomatic and almost impossible to detect in routine examinations
- Rapid, non-invasive diagnostic techniques to detect molecular signatures of retinal injuries are needed.

Hypothesis

- Laser injury causes photoreceptor and RPE cell death and violates the Blood Retina Barrier
- Protein from damaged cells can leak through the damaged blood retina barrier into the systemic circulation
- These proteins can be detected by proteomics in body fluids such as serum and saliva
- These proteins can be used as biomarkers for detection of laser induced retinal injuries.

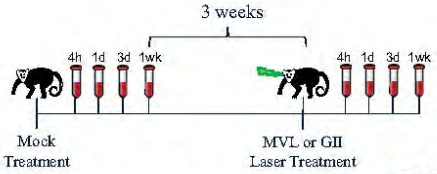
Approach to Laser Injury Biomarker Discovery



Purpose: Identify any statistically significant proteomic changes in response to laser injury, by LC-MS/MS and spectral counting.

Experimental Design

- Animal use protocol approved by USAF Animal Research Programs; proposal #AFOSR-2007-2003A
- 2 Groups, Paired-Control Study
  - Minimal Visible Lesion (MVL), mild; n=6
  - Grade II Lesion (GII), moderate; n=6



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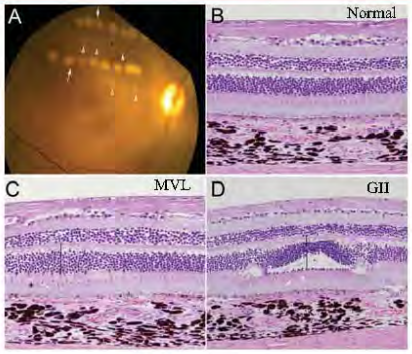
Establishment of Laser Injury



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Sample Processing

- Serum samples were fractionated by isoelectric focusing from pH 3-10.
- Fractionated serum was polymerized into 15% acrylamide gel pieces.
- Gel pieces were digested with trypsin and peptides were extracted.
- Dried samples were resuspended and desalted using a C18 ZipTip column (Millipore).
- Automated nano-flow HPLC-tandem mass spectrometry (LC-MS/MS) was performed.
- Data was blasted against the Uniprot macaque database.

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Data Analysis

- Spectral counting was used to determine relative protein abundances.
- Stringent data filtering and statistical analysis
  - Normalized p-value using G-test
  - p-value adjusted by Holm-Sidak method
  - Proteins retained if:
    - Adjusted p-value < 0.05
    - Scan count ratio > 2.0
    - Occur in at least 50% of laser treated samples
- Minimized rate of false identification and increased confidence in biomarker candidates

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Results: MVL Serum

Time Post-Treatment	Macaca mulatta UniprotID	Human Homolog Gene ID	Protein Description	Number Samples w/ Positive Detection Control (n=6)	Number Samples w/ Positive Detection Treated (n=6)	Normalized Scan Count Ratio (Treated/Control)	Holm-Sidak Adjusted p-Value
4 Hours	Q6X9L5	721	Complement factor (C4)	6	6	2.7780	0.019637
	Q8TUC6	1138	Nicotinic receptor alpha 5 subunit (CHRNA5)	0	5	-	0.003849
	Q28864	7035	Tissue factor pathway inhibitor (TFPI)	0	4	-	0.029670
	B0JDR3	3106	MHC class I antigen (HLA-B)	0	3	-	0.001778
1 Day	B1NL87	64816	Cytochrome P450, 3A43 (CYP3A43)	3	6	6.2458	0.013226
	A6XEX3	146	Alpha-1D adrenoceptor (ADRA1D)	4	5	6.0684	0.00E+00
	P47899	153	Beta-1 adrenergic receptor (ADRB1)	3	4	4.3532	0.000097
	Q61M81	5514	Protein phosphatase 1, regulatory subunit 1C (PPP1R1C)	5	6	4.3240	4.41E-07
	B8Y6V0	51311	Toll-like receptor 8 (TLR8)	0	6	2.2290	0.00E+00
	Q8HYG1	6362	C-C motif chemokine 5 (CCL5)	0	6	[illegible]	0.000036
	Q8YAU2	51182	Caveolin (GRBN)	0	6		0.016833
	Q4G3V3	793	28kDa calbindin 1 (CALB1)	0	6		0.041627
3 Days	Q6GKV9	54429	Taste receptor type 2 (TAS2R5)	2	5	5.0061	0.032944
	B0S4P2	148	Alpha-1A adrenoceptor (ADRA1A)	4	6	5.2097	0.011880
	Q6N143	7297	Tyrosine kinase-2 (TYK2)	6	6	5.2057	0.021489
1 Week	Q28500	5104	Protein C inhibitor (SERPINA5)	1	6	19.4742	0.008141
	P16003	920	T-cell surface glycoprotein (CD4)	6	6	6.5551	3.88E-11
	Q9Z198	6402	L-selectin (SELL)	4	6	4.2801	0.000209
	A4Q687	3126	MHC class II antigen (HLA-DRB4)	2	6	3.6807	0.000548
	Q8MK11	3802	Killer immunoglobulin-like receptor (KIR2DL1)	0	5	-	0.000046
	Q37AI7	8777	Multiple PDZ domain protein (MPDZ)	0	3	-	0.027785

Results: MVL Serum

Time Post Treatment	Macaca mulatta UniprotID	Human Homolog Gene ID	Protein Description	Number Samples w/ Positive Detection Control (n=6)	Number Samples w/ Positive Detection Treated (n=6)	Normalized Scan Count Ratio (Treated/Control)	Holm-Sidak Adjusted p-Value
4 Hours	Q5SPT9	3875	Keratin 18 (CK18)	1	6	16.8935	0.000753
	Q3YAQ9	5230	Phosphoglycerate kinase 1 (PGK1)	5	6	5.0837	0.000309
	Q5WNP0	2525	Lewis alpha-3-fucosyltransferase (FUT3)	6	6	2.2793	0.002356
	Q1HKZ4	1969	Ephrin receptor A2 (EPHA2)	6	6	2.2169	0.003633
1 Day	Q3YAQ9	5230	Phosphoglycerate kinase 1 (PGK1)	2	6	24.6994	2.62E-13
	Q5SPT9	3875	Keratin 18 (CK18)	2	6	22.7121	3.42E-15
	Q5WNP0	2525	Lewis alpha-3-fucosyltransferase (FUT3)	5	6	15.3307	0.000450
	Q1HKZ4	1969	Ephrin receptor A2 (EPHA2)	6	6	2.7874	0.000187

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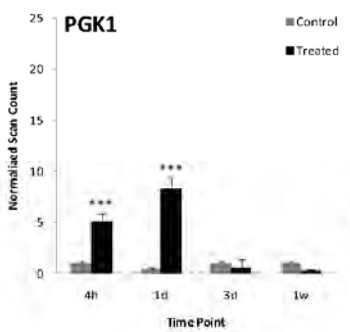
PGK1



- Glycolytic enzyme, abundant in retina
- Upregulated in response to hypoxia
- Released as result of retina tissue damage?

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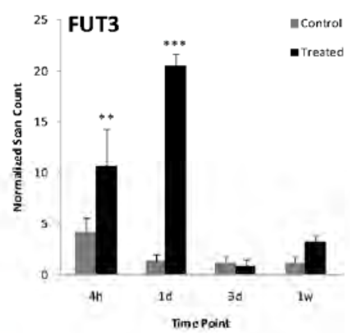
FUT3



- Increased activity with inflammation, neural differentiation, cancer.
- Lewis antigen (glycolipid) synthesized by exocrine epithelial cells (RPE?)
- Glycolipids are ligands for E-selectin receptors.

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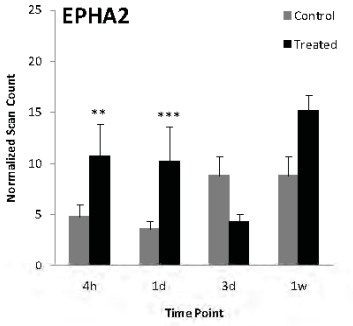
EPHA2



- Expressed in retinal ganglion cells.
- Increased expression of ephrin receptors in brain and retina following laser injury.

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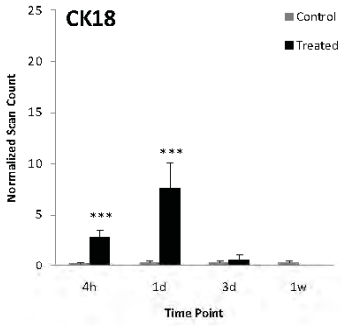
CK18



- Expressed in simple epithelial cells (RPE marker).
- Cleaved by caspase early in apoptosis.
- Fragments have been detected in serum as useful markers of cell death.

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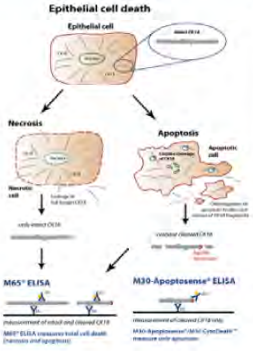
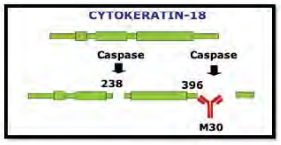
CK18 Detection





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CK18 Detection Detail

4 hr SERUM

	PEPTIDES DETECTED	MOCK 61010	61682	61924	70040	71142	71406	TOTAL	LASER 61010	61682	61924	70040	71142	71406	TOTAL
Cytokeratin 18	VKMEAEIATDR	1	0	0	0	0	0	1	2	1	6	1	6	4	20
	QAQEYEALLNIKVK	0	0	0	0	0	0	0	1	0	0	0	5	4	10
	RTFQSLPINLDSMRNLK	0	0	0	0	0	0	0	0	0	0	2	0	0	2
	ASLENSLR	0	0	0	0	0	0	0	1	0	0	1	0	0	2
	ACYDEMAR	0	0	0	0	0	0	0	0	0	0	0	1	0	1
	IMVDRAQYDEMARKNR	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	TOTALS	1	0	0	0	0	0	1	4	1	6	5	12	8	35

1 d SERUM

	PEPTIDES DETECTED	MOCK 61010	61682	61924	70040	71142	71406	TOTAL	LASER 61010	61682	61924	70040	71142	71406	TOTAL
Cytokeratin 18	VKMEAEIATDR	0	0	0	0	0	0	0	7	27	4	0	13	6	57
	QAQEYEALLNIKVK	0	0	1	0	0	0	1	5	6	2	1	2	2	21
	RTFQSLPINLDSMRNLK	0	0	0	0	0	1	1	1	0	0	0	0	0	1
	ASLENSLR	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	ACYDEMAR	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IMVDRAQYDEMARKNR	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	TOTALS	0	0	1	0	0	1	2	13	33	7	1	16	10	80

MSFTTRSTFSTNYRSLGSVQAPSYGARPVSSAASVYAGAGGSGSRISVSRSTSFRGGMGS
GSLATGIAGGLAGMGGIQNEKETMQSLNDRLASYLDRVRSLETENRRLESKIREHLEKKG
PQVRDWSHYFKIIEDLRAQIFANTVDNARIVLQIDNARLAADDFRVKYETELAMRQSVEN
DIHGLRKVIDDTNITRLQLETEIEALKEELLFMKKNHEEEVKGLQAQIASSGLTVEV*DAPK
SQDLAKIMVDIRAQYDEMARKNREKLDKYWSQQIEESTTVVTTQSAEVGAAETTLTEL
RRTFQSLPINLDSMRNLIKASLENSLREVEARYALQMEQLNGILIHLESELAQTRTEGQKL
AQEYEALNIKVKMEAEIATDRCLEDGEEFNLGLDALP*DENLNTQTQKTTTERRVDGKRVVS
ETNDTKVLRH *Caspase Cleavage Site (Apoptotic Simple Epithelial Cells- RPE)

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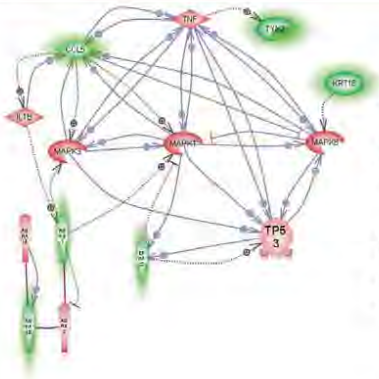
Theoretical Active Pathways



- Based on MVL serum proteomic results
- 6 MVL proteins map to pathway related to TNF-induced apoptosis
- Assays for KRT18 look at presence of fragments in serum due to RPE cell death

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Results: GII Serum

Time Post-Treatment	Macaca mulatta Uniprot ID	Human Homolog Gene ID	Protein Description	Number Samples w/ Positive Detection		Normalized Scan Count Ratio (Treated/Control)	Holm-Sidak Adjusted p Value
				Control (n=6)	Treated (n=6)		
4 Hours	A4QZT4	5266	Elafin preproprotein (PI3)	4	3	5.1679	0.000610
	Q8MQJ7	22914	Killer cell lectin like receptor (KLRK1)	6	6	4.4774	0.000000
	Q8WWN8	6015	E3 ubiquitin protein ligase (RNF41)	6	6	2.1795	0.014974
	Q4JGW4	22889	Regulating synaptic membrane exocytosis 1 (RIMS1)	6	6	2.0294	0.000001
	Q8MC11	3805	Killer immunoglobulin like receptor (KIR2DL4)	0	3		0.001170
1 Day	A1JUM9	3106	MHC class I antigen (HLA-B)	1	4	8.7950	0.017129
	Q2YAV1	122169	Senescence downregulated leu1 like (LEO1)	5	6	4.9732	0.000000
	A5FEZ5	3106	MHC class I antigen (HLA-B)	5	3	4.2168	0.000811
	Q6UX28	10521	DEAD box polypeptide 17 (DDX17)	6	5	3.3807	1.63E-09
	Q9M9G4	8764	Herpesvirus entry mediator (TNFRSF14)	0	4		0.003666
	Q8HZS8	64581	C type lectin domain family 7 member A (CLEC7A)	0	5		0.019497
3 Days	Q2YAV0	3921	Ribosomal protein SA (RPSA)	5	6	6.2766	0.000000
	Q2YS74	150000	ATP binding cassette transporter 13 (ABCC13)	6	5	5.5719	0.000000
	Q546U1	2891	Glutamate receptor subunit 2 (GRIA2)	3	4	4.9404	0.018928
	Q6UX28	10521	DEAD box polypeptide 17 (DDX17)	5	6	4.1664	1.02E-09
	Q5TMS3	136544	Tripartite motif containing 40 (TRIM40)	0	5		0.010417
	Q95M40	4023	Lipoprotein lipase (LPL)	0	4		0.010417
	P30201	4069	Lysozyme C (LYZ)	0	4		0.024736
1 Week	Q2HT70	5304	Prolactin inducted protein (PIP)	1	3	43.8799	0.000000
	A2D668	11244	Zinc fingers and homeoboxes 1 (ZHX1)	4	4	5.5721	0.003992
	Q8WWM4	7923	Hydroxysteroid (17 beta) dehydrogenase 8 (HSD17B8)	0	4		0.021746

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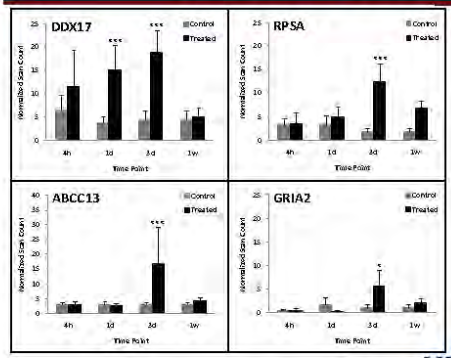
Results: GII Serum



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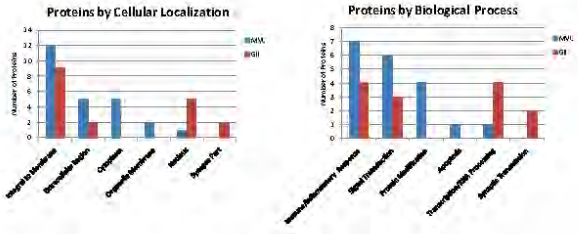
Gene Ontology



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Conclusions

- Model of laser injury for GII and MVL was established in non-human primates.
- Panels of candidate protein biomarkers in response to retinal injury were identified.
- This work was recently published:
  - *Novel serum proteomic signatures in a non-human primate model of retinal injury*. Dunmire JJ, Bouhenni R, Hart ML, Wakim BT, Chomyk AM, Scott SE, Nakamura H, Edward DP. Mol Vis. 2011 Mar 23;17:779-91. PMID: 21527995

Next Steps

- Investigate individual proteins
- Identify "best" diagnostic panel of biomarkers
- Develop immunoassays

Acknowledgments

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Sensors for Monitoring Laser Radiation Exposure

Sensing Strategies, Inc

Dr. Richard Preston

In response to the growing use of lasers in military applications, AF/SGR has developed novel laser sensors to detect and characterize laser radiation exposures. The sensors can be used for occupational health purposes in domestic testing or for force protection in tactical applications. Two types of laser sensors have been fabricated and tested. The first is called the Personnel Protection Sensor (PPS) which is designed to detect pulsed lasers in the 400-1100 nm spectral range. The sensor provides live feedback regarding the exposure levels and indicates if protective eye wear will be effective in preventing injury. The PPS is battery operated and can be run for up to seven hours to log exposures during domestic testing or in ground or flight operations in tactical engagements. The second type of sensor is called the Geolocation Sensor and it characterizes both pulsed and CW lasers. This sensor provides more detailed data on the laser radiation and explicitly measures wavelength and angle of arrival. The Geolocation Sensor is larger in size than the PPS and requires external power to operate. This talk will describe the sensors and present sample test data. AF/SGR welcomes organizations interested in borrowing the hardware for new test applications. AF/SGR will provide test planning consultation with potential users and provide subject matter experts to assist in data analysis if needed.

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AF/SGR Research: Sensors for Monitoring Laser Radiation Exposure

Presented by
Dr. Richard Preston, SSI President
91 Route 31 North, Pennington, NJ 08534

Support provided under AF/SGR program under subcontract to University Of Illinois, Chicago

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The Laser Threat to Commercial Aircraft is Growing

Worldwide laser incidents are increasing in number and sophistication

Commercially available lasers are increasing in power and becoming more portable

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Why Consider Laser Warning Technology?

Safety Zone Compliance

Medical Evaluation

- Aircrew flight readiness
- Additional testing or treatment warranted

Forensic Data

- Provide evidence for trials
- Understand trends
- Identify new threats
- Produce a quantitative data base
- Impact projections

Situational Awareness

- Hazard or annoyance
- Guide interception of perpetrator
- Utility of protection

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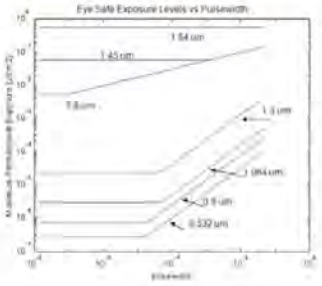
Options for Laser Warning Sensor Deployment

- At or near the target
  - In-cockpit
  - On-person
  - Integrated into vehicle/aircraft
- Away from the target
  - Ground or tower-based
  - Neighboring vehicle/aircraft
- Additional factors:
  - Fixed location vs. portable
  - "Tethered" to power/network vs. stand-alone
  - User-operated vs. autonomous
  - Warning vs. recording (or both)

Different Laser Technologies Pose Different Threats!

- ANSI Standard MPE calculation dependencies
  - Wavelength
  - Pulsewidth
  - PRF
  - Exposure duration
- Bottom Line
  - Damage is much easier to achieve with pulsed lasers
  - Visible lasers are a more serious threat than near-IR



Commercially Available Lasers and Typical Exposures

- Wicked Lasers, Inc. (~$3K)
  - 300 mW, 0.5 mrad, 532 nm
  - 1.5×10^{-5} W/cm² at 3 km
- Well below eye hazard level but very high psychological impact
- Higher power lasers (10W) available commercially as well
- Quantel Brilliant B (~$35K)
  - ~1J/pulse, 7 ns, 0.3 mrad div
  - 28 km nominal ocular hazard distance

How Should Laser Warning Effectiveness be Evaluated?

- Provide technical parameters that are relevant to the requirement
  - For example, to be useful for medical purposes, sensors must report wavelength, amplitude, pulse characteristics
  - Many existing LWR receivers do not detect CW radiation or characterize source as needed for medical and protection purposes
- Some current laser sensors only detect at hazardous levels
  - Field tests show operators want detection thresholds <0.0001× MPE
  - Don't be fooled by argument that below-hazardous exposures should be ignored
- Worthy questions that a LWR should help to answer:
  - What are the levels of current exposures?
  - Are the exposures and techniques changing over time?
  - Is there anything not visible but potentially hazardous buried in exposures?
  - Do I have the right eyewear if needed?

How is Data from Laser Warning Systems Utilized?

- Provides immediate warning to aircrew upon hazard condition
- Provides specific instruction on corrective action (e.g., deploy eyewear, and what type)
- Records detailed event characteristics data for later analyses

In Cockpit Feedback

Assessment of Forensic Data

Warning Plus Recommended Countermeasure

Personnel Protection Sensor

Description
- Designed to warn users about potentially hazardous short-pulse lasers
- Sensor warns user, reports/records level of exposure, and indicates effectiveness of laser eyewear

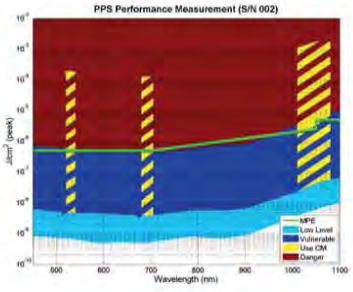
- Rechargeable battery; >7 hours operation
- <1 lb

Technical Specifications
- Spectral range = 400-1100 nm
- Field of view = 110°

Data Products
- Warning messages: "Vulnerable", "Danger", and "Use Countermeasure" (real time display)
- Retrieved file contains GPS time and position where events occurred, and event characterization
  - In-band or out-of band
  - Laser PRF and brightness

Performance Characterization

- Colored regions indicate wide dynamic range of sensor operation
- Performance measured using a wavelength-tunable short-pulse laser (OPO) over a wide range of fluences
- Green line is MPE
- Report lasers *before* they reach a hazard level (indicates "vulnerable" rather than "hazard")
- Low level indication also reported (cyan region) to support situational awareness



PPS Lab Demo: In-Band and Out-of-Band Threats

PPS Sensor Loan To 71 RQS (Moody AFB)

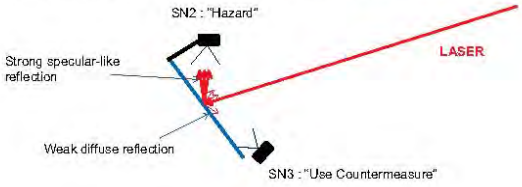
- Two PPS sensors loaned to Moody AFB personnel (Captain Lammens)
  - Obtain operator feedback on functional and performance characteristics
- Flight sorties carried out but no laser detections occurred (as expected)
  - Some false positives on runway near radar
  - SSI working to design package mods to reduce susceptibility
- SSI suggested use of PPS to measure potential eye hazards near laser designator boresight target

PPS Sensor use in Range Safety Application

- PPS sensor installed on and near target board to test for hazardous levels
- In-beam signals confirmed eye hazardous

Target splash test – 26 May '11

- PPS sensors positioned to detect reflections from designator target board
- Sensor readings indicate LEP should be used in target vicinity (they aren't)
- Additional concern for specular reflections
- Sensors also deployed to look for "overshoots"; none seen on 5/28



Moody Deployment Summary

- Useful experience gained in sensor operation by flight crews
  - Expect debrief of Moody personnel in August/September time frame
- Useful data collected on target splash in active area of USAFRICOM
- Will write data summary report to explain utility of activity

Multi-threat Laser Warning Sensor

Description
- Designed to provide broad coverage of multiple types of lasers
- Detect, characterize, and record CW and pulsed lasers at tactically relevant ranges
- For use in ground-based or airborne platforms

Technical Specifications
- Spectral range = 400-1700 nm
- Field of view = 120°
- Pulsed and CW lasers
- Multiple lasers simultaneously (laser cocktails)
- Can cue countermeasures or high resolution imager

- User operated or stand-alone
- Option for networkability

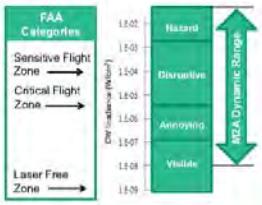
Data Products
- Calibrated amplitude (power/energy density), wavelength, angle of arrival, temporal properties (short pulse indication, PRF)
- All data stamped with GPS time and position
- Can independently characterize simultaneous events

Sensor Performance Good for DOD/LE/Commercial Problems

Threat characterization issues

- SSI experience shows operators want detection/alerting at very low irradiance levels
- Problem space spans huge dynamic range
- Scintillation makes CW lasers appear pulsed (important safety distinction)

Platforms

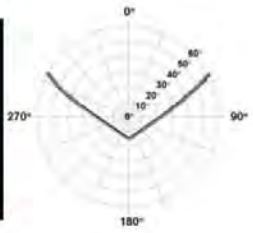


SSI Sensor Data Products

- Irradiance (W/cm²) and/or fluence (J/cm²) (depends on source type)
- Wavelength
- Pulse repetition frequency
- GPS location and time-stamp on hits
- Real-time feedback/cueing
- Data files stored for analysis

M2A Lab Testing Example

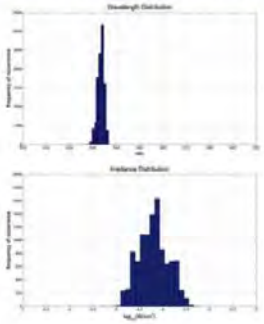
- Eye safe CW green (532 nm) laser (~ 100 μW/cm²)
- Sensor scanned in two dimensions with dark background
- Angle of incidence is reported in real time: reported angle (dark blue) vs. ground truth (light green) is shown in the plot



M2A Outdoor Operation Example

Data from Outdoor Operation

- Eye safe CW green (532 nm) laser (~ 50 μW/cm²)
- Sensor scanned in two dimensions with very bright background (sun in FOV)
- Aggregate wavelength and irradiance distributions shown at right



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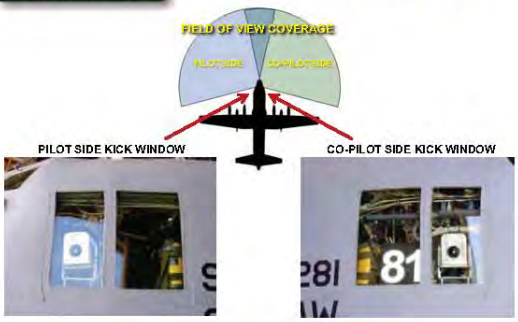
Flight Test Configuration



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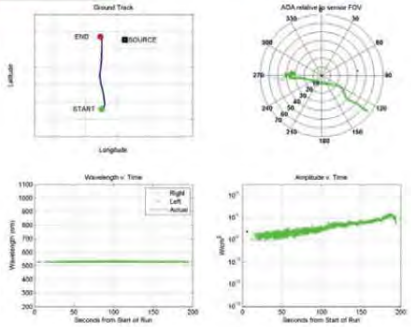
Sample Data Products from Flight Test



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Cursor on Target Demo: *Teaming with AF-A3*

Objective of Test/Demonstration

- Collect laser data and send live over radio link into CoT system
- Data goes to operators on Falcon View and to medical personnel at MOCC
- Data linked to other users from MOCC

Technical Approach

- Define data fields for dissemination and produce CoT compatible format
- Modify Geolocation sensor prototype
- Contract with naval Post Graduate School (NPS)
- NPS-owned CoT surrogate network

Task	Feb	Mar	Apr	May	June	July	Aug	Sep
Kickoff	▲							
Data def.		—						
Sensor mods			—	—	—			
Test planning				—	—	—		
Lab demo						▲		
Field test							—	
Report								—

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CoT Data Products

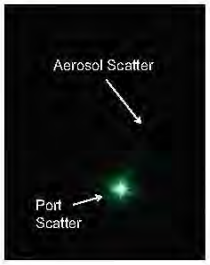
- Pulsed Lasers
  - Exposure relative to MPE
  - PRF
  - Eye safe RF
- CW Lasers
  - Wavelength
  - Amplitude
- Time of event
- Location of sensor

CoT Demonstration Impact

- CoT may be the best way to move data quickly in-theater
- Good demonstration could lead to incorporation of CoT in other SGR delivered hardware
- There will be a need to coordinate with operators and data users for more complete CoT laser threat definition
  - Participate in CoT working group to establish standards

Off-Axis Detection Investigation

- Off-axis scatter mechanisms can be used to detect lasers when someone else is the target
- Signals many orders of magnitude weaker
- Sensor design is different (larger optics and narrower FOVs)
- Demonstrations carried out under SSI SBIR Phase II contract
  - Pulsed designators
  - High power CW



Off-Axis Detection Applications

- Range Safety (LOHAZ)
  - Alert range safety officer if beam leaves safe corridor
  - Provide total energy budget management to prove tests were conducted safely
- Force Protection (Laser Sentry)
  - Detect lasers being used to target friendly forces
  - Forward deployed airbases, convoys

AF/SGR Off-Axis Detection Opportunity

- AF/SGR providing $40K for Eglin AFB to run one week test for on and off-axis laser detection
- Eglin AFB will provide test range with forward airbase mock-up
- AATC will provide one Special Ops Forces Laser Aided Marker
- Test Objectives
  - Simulate force protection mission on test area
  - "Optical fence" monitoring of pulsed laser testing
- Expected outcome
  - Demonstration of sensor concept for improving safe range operations and extended area laser threat monitoring
  - SBIR Phase 3 a contractual option if building a deployable prototype for field demonstration (CENTCOM) is desired

Summary

- SGR has successfully developed sensors suitable for characterizing laser radiation sources
- SGR continues to coordinate with operators to get prototype hardware fielded for user-feedback and lessons learned
  - Range safety
  - Battlefield
- Data management and dissemination remains key topic of interest so data ends up in the right hands/organizations
- SGR will continue applying sensors to occupational health and force protection missions

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Gene Expression Profile of Jurkat Cells Exposed to High-Power Terahertz Radiation

711 HPW/RHDR

1Lt Jessica Grundt

Terahertz (THz) radiation sources are now being used in a various military, defense, and medical applications. Widespread employment of these new applications has prompted concerns regarding the potential health effects associated with THz radiation. A source for these concerns stems from results of recent studies which provide evidence that THz radiation can couple directly to biological macromolecules (lipids, DNA, proteins) causing localized effects affecting gene transcriptional processes. In this work, we hypothesized that if THz radiation does cause direct damage to biological macromolecules, then THz-exposed cells may express a specific gene expression profile indicative of this unique damage. To test this hypothesis, Jurkat cells were irradiated with a molecular gas THz laser (2.52 THz, 636 mWcm-2, durations: 5, 10, 20, 30, 40, or 50 min). Cellular viability was assessed 24 h post-exposure using conventional MTT assays, and gene expression profiles were evaluated 4 h post-exposure using mRNA microarrays gene chips. Comparable analyses were also performed for hyperthermic (bulk heated) positive controls (44°C for 40 min). We found that many of the genes that were upregulated in the THz-exposed samples were also expressed in the thermal controls; however, several genes were only expressed in the THz exposure group. Interestingly, these target genes are known to function in the regulation of cellular proliferation, membrane repair, and transcriptional processes. These results suggest that THz radiation may couple to biological macromolecules resulting in direct effects, which do not appear to be fully attributable the temperature rise generated during exposures (i.e. conventional thermal effects).

AFRL
THE AIR FORCE RESEARCH LABORATORY
LEAD • DISCOVER • DEVELOP • DELIVER

GENE EXPRESSION PROFILE OF JURKAT CELLS EXPOSED TO HIGH-POWER TERAHERTZ RADIATION

3 Aug 2011

1st Lt Jessica E. Grundt
Directed Energy Bioeffects Division
Air Force Research Laboratory

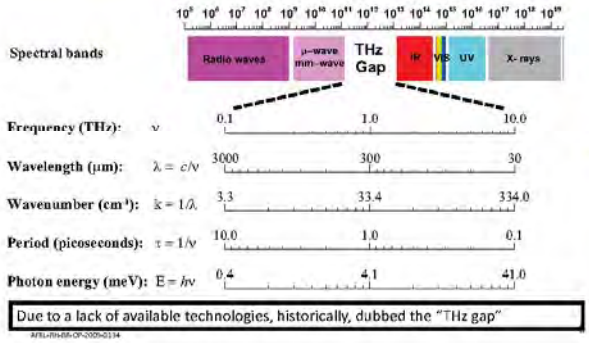
DISTRIBUTION STATEMENT A. Approved for public distribution; PA# 11-488

Talk Outline

- Terahertz Introduction
- Motivation for THz Bioeffects Research
- Recent Experiment
- Results
- Summary and Impact
- Acknowledgements/Questions

Terahertz (THz) Introduction

Electromagnetic spectrum



Due to a lack of available technologies, historically, dubbed the "THz gap"

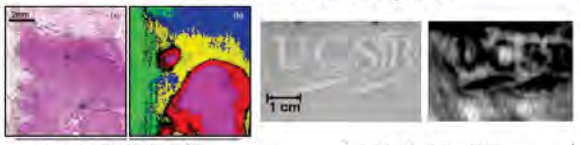
AFRL-RH-BR-OP-2005-0134

THz Applications that Exploit These Unique Properties

Military, Security, & Defense

Clery *Science* (2002) Karpowicz *APL* (2005) L3 ATD at Airports Wilmink et al. *JBO* (2011)

Medicine: Cancer & Burn Diagnosis



Brun et al. *PhysMedBio* (2010) Taylor et al. *Opt Letters* (2008)

Motivation for Carrying Out Our THz Bioeffects Studies

1. **Not well characterized: < 40 THz bioeffects studies conducted to date**
   Pakhomov *et al.* BEMS 1998. 13 years ago > 300 at mmwave frequencies
   Wilmink GJ *et al.* Invited Review Article, Int J THz, 2011.
2. **Korenstein-Ilan *et al.* (2008). Radiation Research.**
   "Terahertz Radiation Increases Genomic Instability in Human Lymphocytes"
   THz radiation induces morphological changes indicative of genomic instability.
3. **Alexandrov *et al.* (2010). Physics Letters A.**
   "DNA Breathing Dynamics in the Presence of a Terahertz Field"
   THz radiation may resonate with natural breathing mode of double stranded DNA.
4. **Most importantly → The Air Force Surgeon General told us to**

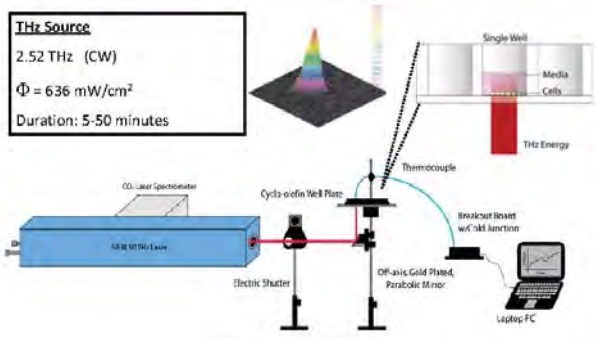
Air Force Surgeon General

Project goal: Characterize the response of human dermal fibroblasts exposed to THz radiation, heat, and genotoxic stress

Our Approach

1. Expose Jurkat cells to 2.52 THz radiation or bulk heating
2. Empirical and computational dosimetry
3. MTT viability assays
4. Microarray gene chips (mRNA & microRNA) to identify signature gene expression patterns
5. Compare and contrast gene data
6. Bioinformatics
   - Identify putative biomarkers and pathways
   - Location of functional activity of biomarkers (i.e., nucleus, lipid membrane, cytosol)
7. Validate expression of key genes using qPCR

1 Setup to irradiate Jurkat cells with 2.52 THz radiation



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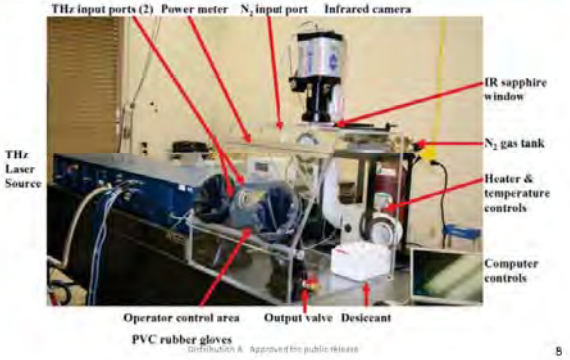
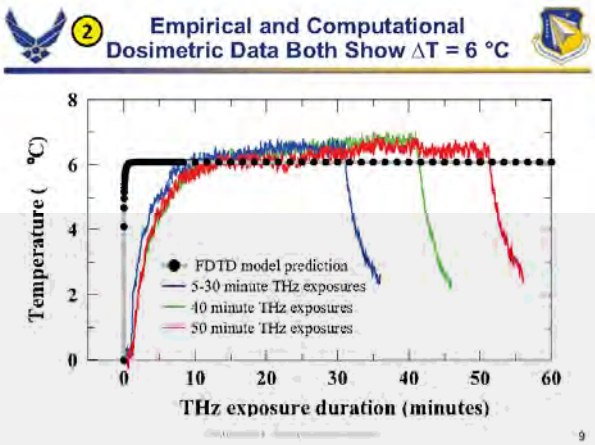
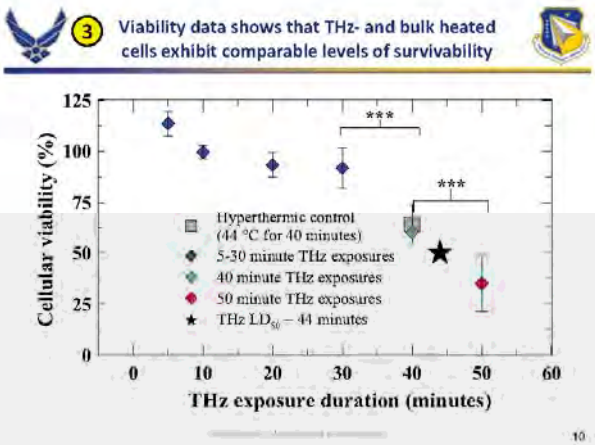
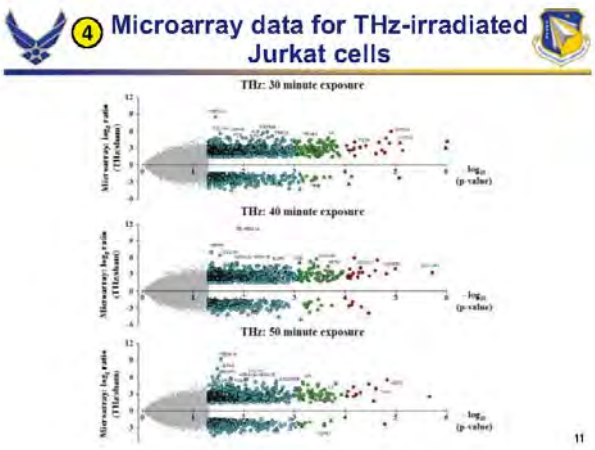
1 Image of our custom temperature controlled exposure enclosure system

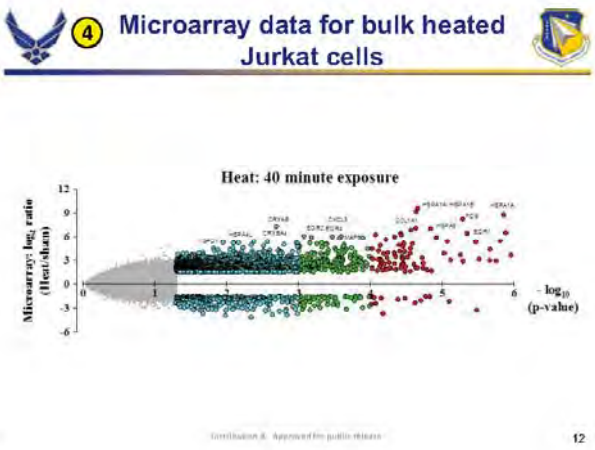


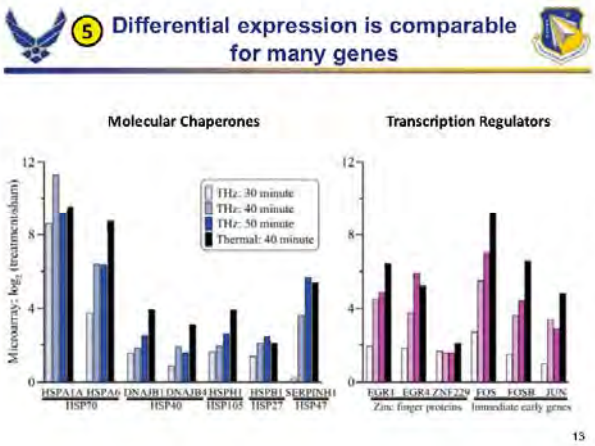
Distribution A: Approved for public release. 8

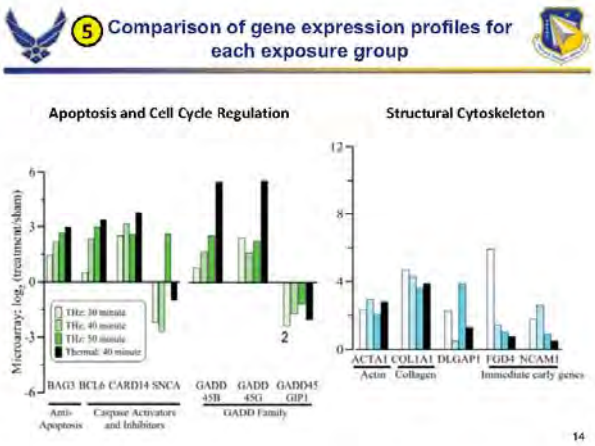

2 Empirical and Computational Dosimetric Data Both Show ΔT = 6 °C
Temperature (°C)
THz exposure duration (minutes)
FDTD model prediction
5-30 minute THz exposures
40 minute THz exposures
50 minute THz exposures
9

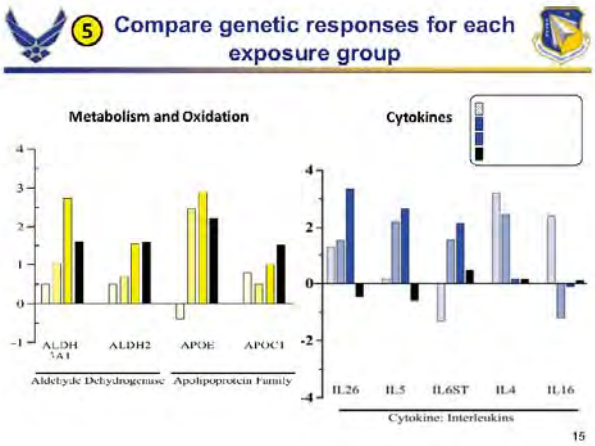

3 Viability data shows that THz- and bulk heated cells exhibit comparable levels of survivability
Cellular viability (%)
THz exposure duration (minutes)
***
***
Hyperthermic control (44 °C for 40 minutes)
5-30 minute THz exposures
40 minute THz exposures
50 minute THz exposures
THz LD50 – 44 minutes
10

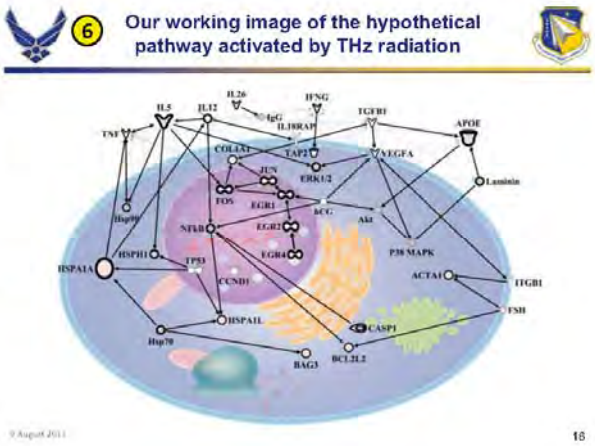

4 Microarray data for THz-irradiated Jurkat cells
THz: 30 minute exposure
THz: 40 minute exposure
THz: 50 minute exposure
11


4 Microarray data for bulk heated Jurkat cells
Heat: 40 minute exposure
12

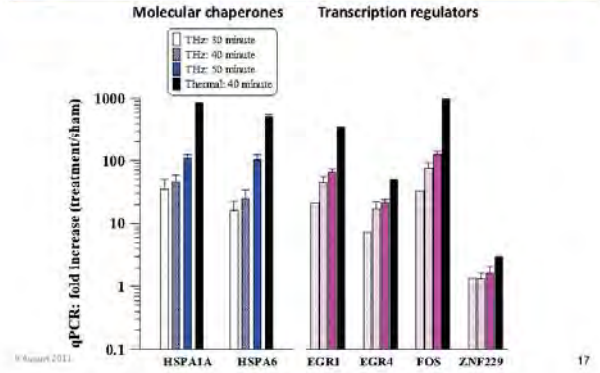

5 Differential expression is comparable for many genes
Molecular Chaperones
Transcription Regulators
Microarray: log2 (treatment/sham)
THz: 30 minute
THz: 40 minute
THz: 50 minute
Thermal: 40 minute
HSPA1A HSPA6 DNAJB1 DNAJB4 HSPH1 HSPB1 SERPINH1
HSP70 HSP40 HSP105 HSP27 HSP47
EGR1 EGR4 ZNF229 FOS FOSB JUN
Zinc finger proteins Immediate early genes
13


5 Comparison of gene expression profiles for each exposure group
Apoptosis and Cell Cycle Regulation
Structural Cytoskeleton
Microarray: log2 (treatment/sham)
THz: 30 minute
THz: 40 minute
THz: 50 minute
Thermal: 40 minute
BAG3 BCL6 CARD14 SNCA
GADD 45B GADD 45G GADD45 GIP1
ACTA1 COL1A1 DLGAP1 FGD4 NCAM1
Actin Collagen Immediate early genes
Anti-Apoptosis Caspase Activators and Inhibitors
GADD Family
14


5 Compare genetic responses for each exposure group
Metabolism and Oxidation
Cytokines
ALDH 5A1 ALDH2 APOE APOC1
Aldehyde Dehydrogenase Apolipoprotein Family
IL26 IL5 IL6ST IL4 IL16
Cytokine: Interleukins
15

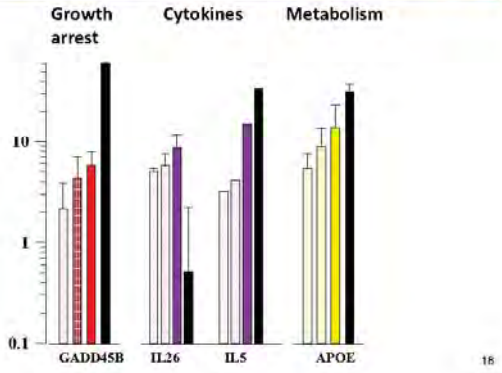

6 Our working image of the hypothetical pathway activated by THz radiation
IL5
IL12
IL26
IFNG
TGFB1
APOE
VEGFA
Laminin
JUN
FOS
EGR1
ERK1/2
NFkB
EGR2
EGR4
HSPH1
HSPA1A
TP53
CCND1
P38 MAPK
ACTA1
ITGB1
FSN
HSPA1L
CASP1
Hsp70
BAG3
BCL2L2
16

7 qPCR Validation data is consistent with microarray data



17

7 qPCR Validation data is consistent with microarray data



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Summary and future directions

Summary

1. In general, human Jurkat cells launch similar transcriptional gene expression profiles when exposed to THz radiation or bulk heat
2. However, several biomarkers appear to be only expressed in THz-exposed samples
   - Interestingly, these genes encode for cytokines and proteins responsible for maintenance of plasma membrane properties

Future directions

- Investigate varying:
  - Cell lines
  - Frequencies
  - Pulsed vs CW

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Acknowledgements

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Department of Defense Biological Threat Responses to the 2009-2010 H1N1 Influenza Outbreak

AF/A5XP

Ms. Calli Levin

Beginning in April 2009 with the outbreak and rapid spread of the H1N1 "swine flu," the world witnessed the potential effects of a bioterrorist attack. While the 2009-2010 H1N1 pandemic was a naturally-occurring disease outbreak and not a deliberate attack, the symptoms, infection rates and response mechanisms associated with the virus could be similar to the impacts of a deliberate biological agent attack. Unlike nuclear or chemical weapons that have clearly identifiable signatures, biological agents may be disseminated covertly, and therefore they may not be identified immediately. The first indication of a biological event could be more numerous-than-expected hospital visits in a particular location (e.g. a military installation), or in a group of people who were in the same location at the same time (e.g. basic combat training). Force health protection planners will be better positioned to respond to future biological events using experience gained during the H1N1 pandemic. It provided the Department of Defense an opportunity to exercise disease containment planning measures and address biological warfare response mechanisms. Seventy-five percent of H1N1 infections worldwide involved those under 30 years of age—a significant statistic for the DoD as more than 66 percent of active duty military personnel are within that age bracket. The H1N1 outbreak prompted the DoD to implement a range of force health protection measures, focusing on social distancing efforts called for in USNORTHCOM CONPLAN 3551, and on vaccination campaigns. This presentation will address the protective measures implemented by the DoD and will present key lessons learned.

Headquarters U.S. Air Force

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DoD Responses to the 2009-2010 H1N1 Influenza Outbreak

Ms. Calli Levin
AF/A5XP
3 Aug 2011

U.S. AIR FORCE

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Agenda

- The Biological Threat Environment
- Why Pandemic Influenza Matters to DoD
- DoD Responses to H1N1
- Lessons Learned/Best Practices

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The Biological Threat

"One of our Greatest Concerns continues to be that a terrorist group or other dangerous group might acquire and employ biological agents…to create casualties greater than September 11."

Former Director of National Intelligence Michael McConnell

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Why PI Matters to DoD (1/2)

- Services are required to respond to and mitigate bio events of operational significance whether naturally occurring or deliberate
  - A5XP is OPR for AF Disease Containment Planning
  - AF Medical career fields will implement plans
- Many bio threat responses—especially medical responses—will be similar in natural and deliberate outbreaks
  - Unlike chemical or nuclear threats, no clear bio signature
  - Outbreak will have same or greater effect on employee absenteeism, school and work closures, distribution of medical/nonmedical countermeasures, mortality rates
  - Bio event may have major effect on mission continuation

H1N1 provided DoD opportunity to exercise bio-threat responses

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Why PI Matters to DoD (2/2)

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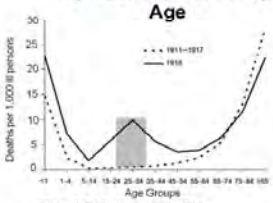
Spanish Flu Deaths by Age



Average Age by Service (2008)

Service	18-21	22-30	31-40	41-50	51-60	Average Age
Army	18.3%	46%	26.5%	7.9%	0.7%	29
Navy	18.5%	48%	25.3%	8.3%	0.8%	29
Marine Corps	26.9%	45%	14%	3.1%	0.2%	26
Air Force	14.4%	48%	28.3%	10%	0.8%	30
Coast Guard	12.2%	46%	27%	12%	1%	30
Total	19.9%	47%	24.8%	8%	0.8%	29

The 1918 Spanish Flu:

- Killed between 20-50 million people
- Killed more US Servicemen than WWI
- Infected 25 percent of the US population

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H1N1 2009 Timeline

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April 26: First cases reported in Mexico, US
April 27: 73 cases in four countries
April 29: World Health Organization (WHO) declares Phase 5 Outbreak (human-to-human spread within one region)
June 11: 17,400 cases in 62 countries; WHO declares Phase 6 Global Outbreak

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Non-Medical Response Measures

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- Social distancing
- Travel restrictions
- AF public affairs health campaigns
  - Stay at home when sick
  - Cover mouth when coughing
  - Wash hands regularly
- Alternate work schedules/telework
- C-BW exercises
- Updating disease containment plans
- Operational risk management

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Medical Response Measures

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- Anti-viral drug stockpile and usage
  - Relenza, Tamiflu stockpiled at medical treatment facilities
- Vaccine procurement and distribution
  - Research and development
  - Prioritization
  - Immunization Campaign
- H1N1 Reporting
  - USNORTHCOM CONPLAN 3551
  - Phases 0/1 – Monthly reporting
  - Includes impact on medical facilities, services, resources

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H1N1 Vaccine Timeline: 2009

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OCTOBER 2009
- First 1.4M doses delivered
- DoD orders additional 1M doses for government civilians and military dependents

SEPTEMBER 2009
President allocates $2.7B to immunization campaigns

AUGUST 2009
DoD purchases 2.7M doses of H1N1 vaccine

JUNE 2009
HHS allocates additional $1B to vaccine R&D

MAY 2009
HHS allocates $1B to vaccine R&D

H1N1 Lessons Learned

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- PI policy guidance during outbreak confusing, not aligned
  - USNORTHCOM, DoD, World Health Organization all different
  - No cutoff mechanism for CONPLAN 3551 reporting
  - No Disease Containment Plan for Headquarters AF
- Anti-viral stockpile difficulties
  - Lack of Memorandum of Understanding with state
  - Unaware of how to request Tamiflu if not part of formulary
- Travel restrictions not standardized across AF or DoD
- Personnel accountability systems not equipped for lengthy event– current systems require one report/event; cannot update
- Employees unable to telework as planned– lack of policies, equipment, and software knowledge hampered employee access

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H1N1 Best Practices

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- Early detection/surveillance
- Vaccination program
- Public Affairs health campaign
- Prior disease containment planning

Immunization Coverage (Mar 10)

(Active Component)	DoD-All*	ARMY	MARINES*	NAVY*	COAST GUARD	AIR FORCE
Seasonal Vaccine	93%	95%	86%	86%	97%	97%
Pandemic Vaccine	84%	88%	77%	76%	92%	90%

*Underestimates due to time lag in reporting systems

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Additional Observations

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- DoD responses to H1N1 were successful
  - Limited operational impact
  - Few military deaths relative to civilian population
  - PI guidance practiced/validated
- Hundreds of lessons learned input into DoD sites—major items being addressed through working groups, task forces
  - PI policy guidance
  - Stockpile issues
  - Telework limitations

What did YOU experience during H1N1?

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QUESTIONS?
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13

Expanding Surge Capacity in Airborne Isolation & Worker Protection During Bioterrorism & Epidemic Response

U.S. Public Health Service

CDC - NIOSH

CAPT Kenneth Mead

Shortages in airborne infection isolation capacity are well documented within the U.S. healthcare system. During an airborne infectious epidemic, non-traditional healthcare environments such as field medical shelters, social service facilities, nursing homes, and quarantine stations, could also require emergency airborne isolation capacity. An affordable method for expedient airborne infection isolation is required to meet emergency surge requirements. The research discussed in this presentation began as an investigation of expedient methods to establish airborne infection isolation within conventional, non-isolation hospital rooms using portable filtration units and common hardware supplies. The research focused enhanced scrutiny on concentration reduction and worker protection, rather than focusing solely upon containment strategies. For the field studies, two airborne isolation configurations were evaluated within each of four Midwestern hospitals. Results revealed the expedient airborne isolation configurations were successful at airborne containment while also providing significant reductions in potential worker exposures. Concentration reduction ratios were 98-99 percent or greater, resulting in workplace protectionfactors several times greater than that assigned for N95 respirators. Subsequent research has expanded the concepts to medical shelters and other alternative-care environments and has begun to investigate adaptations for ambulance interiors. One application even operates off-the-grid in austere environments. The ability to keep response workers healthy should be a paramount consideration when managing an emergency response operation. When combined with the requirement for isolating infectious patients to avoid further disease propagation, the findings of this research effort could have important implications upon U.S. healthcare emergency planning policies.

Expanding Surge Capacity in Airborne Isolation & Worker Protection During Bioterrorism & Epidemic Response

CAPT Kenneth R. Mead, Ph.D., P.E.
Centers for Disease Control and Prevention
National Institute for Occupational Safety & Health
Cincinnati, OH

CDC CENTERS FOR DISEASE CONTROL AND PREVENTION

NIOSH National Institute for Occupational Safety and Health

Isolation as a Control Measure

Expedient Airborne Isolation for Healthcare Facilities During Emergency Epidemic Response

Purpose: To ID & evaluate effective parameters for patient isolation and healthcare worker protection to meet airborne isolation surge requirements during bioterrorism or epidemic emergency events:

Basically looking for a cheap, easy, yet effective method for reducing potential exposures to healthcare workers.

Disclaimers

- *"The findings and conclusions in this presentation are those of the author and do not necessarily represent the views of the Centers for Disease Control and Prevention or the National Institute for Occupational Safety and Health"*
- *"Mention of company names or products does not constitute endorsement by NIOSH"*

Recent Events & Concerns

- Multidrug-resistant tuberculosis (MDR-TB)
- Bioterrorism (Smallpox, Plague…)
- SARS
- Extensively drug resistant TB (XDR-TB)
- Monkeypox
- H5N1
- H1N1
- Extremely Drug Resistant TB (XXDR-TB)
- MRSA, C. Dif., …
- ???

5

Engineered Airborne Infection Isolation (AII) Design Summary*

- Dedicated single-patient room
- At least 12 air changes per hour (ACH) of total ventilation (new construction), including a minimum 2 ACH outside air
- Maintained at negative pressure relative to adjacent areas (minimum delta P of 0.01 inches water gauge or 2.5 Pa) with seams & penetrations sealed
- All air exhausted to outdoors, unless HEPA-filtered and returned to dedicated HVAC system

* Design Guide Sources: CDC, ASHRAE, FGI

The Problem

- Almost 40% of U.S. hospitals lack an engineered AII room. (AHA, 2006)
- Large hospitals typically have a few AIIR's and small hospitals may have 1,
- Essentially NO engineered surge capacity in case of epidemic (natural or intentional)
- Non-hospital medical, social service facilities, and health departments generally lack isolation capabilities
- Cost ~ $30K-$40K per room to construct

Example: Limited Surge Capacity

- Nevada Hospital Association
  - State Survey (2006)
  - 216 AII beds plus 91 bed surge capacity
  - 307 "available" AII beds to serve roughly 2.5 million residents plus an average of over 4 million visitors/month

8

Response Options:
Aren't Always Worker-Friendly

- Patient transfer
- Big-area iso (hot) zones with patient cohorting
- Respirators and surgical masks and traditional patient rooms
- Traditional patient room + Portable HEPA units to get 6-12 ACH of dilution filtration

Limitations of Dilution

- Poor room air mixing adversely impacts removal efficiency
- The airborne pathogen circulates throughout the room
  - *All occupants exposed to "same" concentration*
  - *Increased distribution of surface contamination*
  - *Increased risk of contaminant migration out of the room*
- Shouldn't be used when worker BZ is close to source
- Portable filtration – little guidance on how to deploy

Dilution Wait Times for Desired Removal Efficiency

ACH	Minutes Required for the Desired Removal Efficiency		
	90%	99%	99.9%
2	69	138	207
6	23	46	69
12	12	23	35

Assuming the aerosol source is stopped and a good dilution ventilation design (K=3), it will take 69 minutes (3 x 23) to achieve a 90% dilution of airborne aerosol (90% reduction = protection factor of 10) or 138 min for the "standard" 99% reduction.

three can be assumed for a room with 12 ACH and good air movement).

$$C_2 = C_1 e^{\left[\frac{Q\Delta t}{V}\right]} \qquad \Delta t = -\left(\frac{V}{Q}\right)\ln(C_2 / C_1)$$

Hierarchy of Controls
ranks actions by their likely effectiveness

Listed in order of preference:

- Elimination – eliminates the source of the exposure
- Engineering Controls – uses engineering approaches to contain source and reduce exposures below harmful levels
- Administrative Controls – Uses administrative directives regarding work practice, shift rotations and prophylaxis to limit opportunities for possible harmful exposures
- Personal Protective Equipment - Wearing gloves, gowns, masks, respirators and other PPE appropriate for the hazard

Comment: When it comes to hands-on health care and an airborne infectious disease for which there is not a vaccine, the traditional approach has been to switch immediately to PPE Controls.

Research Scenarios

- Used portable HEPA filtration units like those already found in health care facilities to identify expedient alternative approaches to provide airborne isolation



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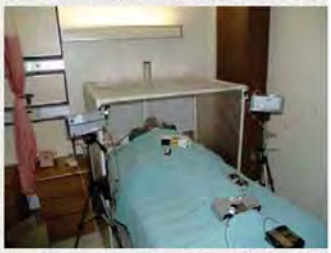
Alternative Approaches

- Reduce volume of contaminated zone (a.k.a. Zone-Within-Zone)
  - Effectively increases ACH w/in inner zone

Alternative Approaches

- Use local control techniques (a.k.a. Ventilated Headboard w/Canopy)
  - Captures and removes contaminant before it has a chance to disperse.
  - Reduces the required time for the overall room to achieve a desired removal efficiency.



Qualitative Smoke Tests

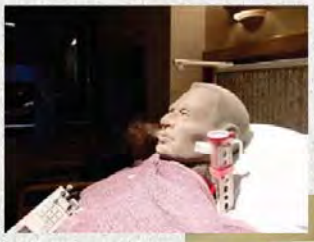
- *"Scientific" hand-held smoke generator*

- *Educational "toy"*

Source (Aerosol) Generation

- Medical Nebulizer
- R.O. H_2O w/ 3 drops ~1.6 *um* polymer microspheres

Aerosol Generation/Measurement



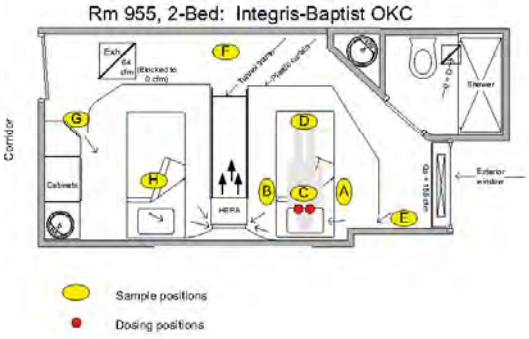
Field Methodology

- The research was performed in multiple healthcare settings not currently engineered for airborne infectious isolation.
- Selected locations were two urban hospitals and two smaller, rural hospitals all within the states of Oklahoma and Kansas.
- Each facility received repetitive evaluations of the two expedient isolation design variations previously identified in the feasibility study.

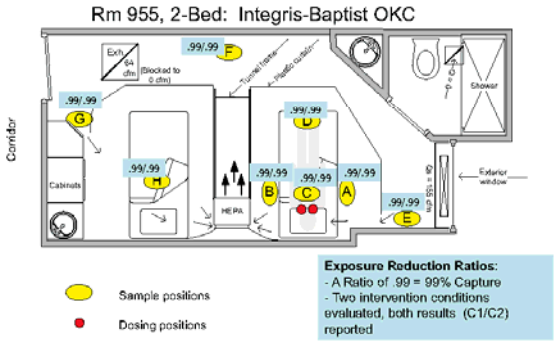
18

Integris Baptist Medical Center
Zone-within-Zone
OKC, OK

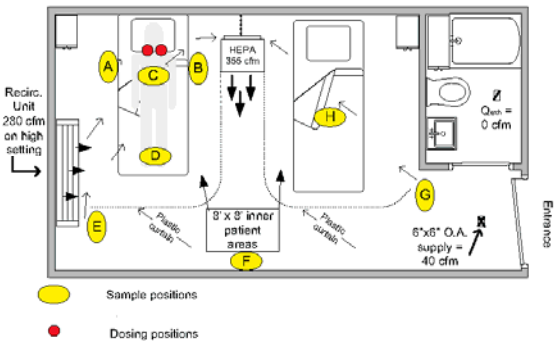
Rm 955, 2-Bed: Integris-Baptist OKC



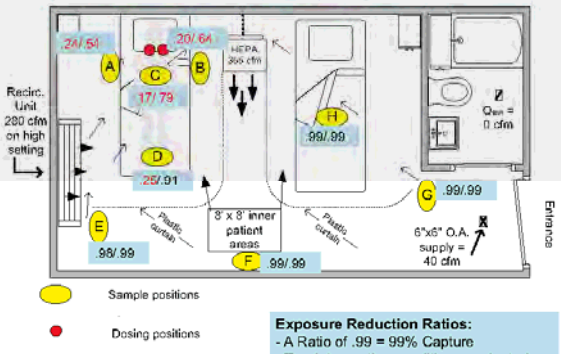
Integris Baptist Medical Center
Zone-within-Zone
OKC, OK



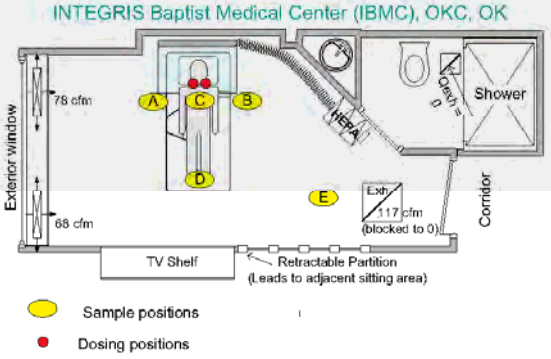
Zone-within-Zone, ST Joseph Memorial Hospital (SJMH), Larned, KS



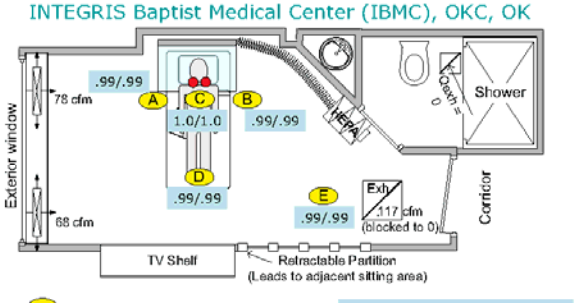
Zone-within-Zone, ST Joseph Memorial Hospital (SJMH), Larned, KS



Ventilated Headboard (1-Bed)
INTEGRIS Baptist Medical Center (IBMC), OKC, OK



Ventilated Headboard (1-Bed)
INTEGRIS Baptist Medical Center (IBMC), OKC, OK



Exposure Reduction Ratios:
-A Ratio of .99 = 99% Capture
1.0 = "Zero" Exposure
-Two test conditions evaluated, results for both (C1/C2) reported

GMRR Summary (lower limits, simultaneously–corrected for $\alpha = 0.10$, in parentheses), **Zone-Within-Zone** (2-Bed) configuration, Gray columns = corner-to-corner dilution flow, White Columns=side-to-side source control flow

(Bold Red font = GMRR <90%)

Hospital / *Sample Pos.*	*VAMC* 2:1	*VAMC* 3:1	*CKMC* 2:1	*CKMC* 3:1	*SJMH* 2:1	*SJMH* 3:1	*IBMC* 2:1	*IBMC* 3:1
HCW-Upstream	**0.134** (**-4.10**)	**0.163** (**-5.65**)	0.998 (0.993)	0.993 (0.971)	**0.241** (**-0.536**)	**0.544** (**0.076**)	0.998 (0.986)	0.998 (0.989)
HCW-Downstream	**-0.767** (na)	**-0.800** (na)	0.928 (na)	0.993 (na)	**0.204** (na)	**0.641** (na)	0.996 (na)	0.999 (na)
Patient chest	na (na)		**0.761** (na)	1.00 (na)	**0.171** (na)	**0.791** (na)	0.998 (na)	0.999 (na)
Patient feet	na (na)		na (na)		**0.247** (**-0.525**)	0.911 **0.821**)	0.999 (0.994)	0.998 0.991)
Outside Gap 1	0.998 (0.991)	0.999 0.989)	0.998 (0.994)	0.993 0.983)	0.984 (0.968)	0.991 0.982)	0.998 (0.987)	0.998 0.991)
Center Room	0.999 (0.994)	0.999 0.991)	0.999 (0.996)	0.998 0.996)	0.996 (0.992)	0.996 0.992)	0.995 (0.970)	0.996 0.979)
Outside Gap 2	0.993 (0.958)	0.997 0.979)	0.999 (0.996)	0.999 0.998)	0.988 (0.965)	0.997 0.989)	0.998 (0.987)	0.997 0.981)
Bed 2	0.987 (0.942)	0.997 0.989)	0.999 (0.996)	0.996 0.991)	0.987 (0.971)	0.991 0.982)	0.998 (0.990)	0.996 0.979)

GMRR Summary (lower limits simultaneously–corrected for α = 0.10 in parentheses), **Ventilated Headboard** (1-Bed) configuration

(Bold Red font = GMRR <90%)

Hospital / *Sample Pos.*	*VAMC* 2:1	*VAMC* 3:1	*CKMC* 2:1	*CKMC* 3:1	*SJMH* 2:1	*SJMH* 3:1	*IBMC* 2:1	*IBMC* 3:1
HCW-RHS	0.987 (0.947)	0.996 0.979)	0.999 (0.996)	0.997 0.991)	0.998 (0.996)	0.997 0.995)	0.998 (0.990)	0.998 0.993)
HCW-LHS	0.997 (0.986)	0.996 0.980)	0.998 (0.995)	0.998 0.993)	0.998 (0.996)	0.998 0.997)	0.999 (0.997)	0.998 0.994)
Patient chest	1.00 (1.00)	1.00 0.998)	0.967 (**0.898**)	0.920 **0.724**)	0.998 (0.997)	0.997 0.995)	1.00 (1.00)	1.00 1.00)
Patient feet	0.995 (0.979)	0.997 0.984)	0.996 (0.989)	0.993 0.977)	0.996 (0.993)	0.997 0.995)	0.998 (0.990)	0.998 0.993)
Center Room	0.997 (0.988)	0.996 0.980)	0.997 (0.990)	0.996 0.985)	0.997 (0.995)	0.998 0.996)	0.999 (0.994)	0.997 0.989)

NEW TERM: Expedient Isolation Protection Factor (EIPF)

- A surrogate measure of the workplace protection
- Analogous to Simulated Workplace Protection Factor (SWPF)used by NIOSH in respirator testing.
- EIPF can be calculated by:

$$EIPF = (1 - GMRR)^{-1.0}$$

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Expedient Isolation Protection Factors (EIPF)

Zone-within-Zone Configuration

- **Inner Zone**:

Corner-to-Corner: EIPFs negligible or neg.

Side-to-Side:

Upstream: Mean EIPF = 308 (143-1000)

DnStream: Mean EIPF = 48 (14 - 1000)

- **Outer Zone**: Mean EIPF = 222 (63-1000)

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Expedient Isolation Protection Factors (EIPF)

Ventilated Headboard Configuration

- GMRRs = 1.0 must be carried out to true value (<1) for EIPF formula to apply
- Across four study sites, Center Room and worker positions:

Mean EIPF = 338* (77-1000)

*>30 times OSHA's N95 PF of 10

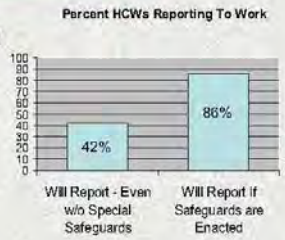
30

Why Is Healthcare Worker (HCW) Protection Important?

- Polling: As few as 24 percent (worse case comb. of willingness and ability) of greater New York HCW's willing to report to work for an infectious airborne epidemic such as SARS[1].
  - Fear regarding personal and family safety were the primary factors.
- Results consistent with Israeli study[2].

However

- Predicted worker reporting increased dramatically if personal safety measures were available[2]!!



References:

1. Journal of Urban Health: Bulletin of the New York Academy of Medicine. 2005;82(3): p.378.
2. Israel Medical Science Journal 1991;27: p. 704

Polling Data vs. Real Events

- Polling data may be optimistic
- 2003 Monkeypox experience
  - Symptoms: Initially present as smallpox

33

Pediatr Infect Dis J. 2003;22:1093–6
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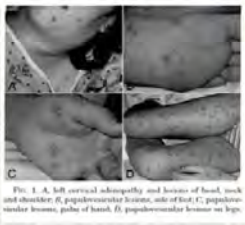
Vol. 22, No. 12
Printed in U.S.A.

A case of severe monkeypox virus disease in an American child: emerging infections and changing professional values

MICHAEL G. ANDERSON, JD, MD, LAWRENCE D. FRENKEL, MD, SCOTT HOMANN, MD AND JENNIFER GUFFEY, MD



"One unexpected complication of the admission was the difficulty in finding nurses and physicians willing to care for the patient. Many declined with the explanation that they had not received smallpox vaccine, and others declined direct patient contact without explanation."

Slide Credit: P.K. Carlton

Ped Infect Dis J, 22:1093-1096, 2003

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Conclusion

- Current guidance does not adequately address isolation response needs at the local level.
- Shortages of isolation capacity may impede the medical response to an emergency
- Current trends in surge iso design do not sufficiently address worker protection issues
- Expedient in-room isolation units employing high-flow HEPA filtration offer alternatives to emergency AII that are:
  - **Affordable** - **Available**
  - **Effective** - **Simple**

Acknowledgements & Gratitude

David Johnson, Daniel Boatright, Nurtan Esmen, Ramkumar Parthasarathy, Margaret Phillips and Robert Lynch

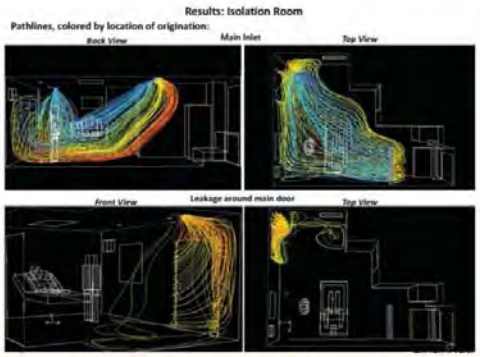
Duane Hammond, Byran Adams, Adam Paberz, Dan Farwick, Stan Shulman, Amy Feng, Mike Gressel, Phil Comp, Beverly Stiles, Tim Cathey, Chris Thomas, Stacey Martin, Nancy Winkelman, Paul Hedrick, Danielle Johnson, John Broaderway, Harry Goett, Barbara Morton, Joe Fernback, Erik Devine, Nick Trifonoff

Central Kansas Medical Center, Great Bend, KS
INTEGRIS Baptist Medical Center, OKC, OK
St. Joseph Memorial Hospital, Larned, KS
VA Medical Center, OKC, OK

Current/Future Activities – continued

- CFD (UC)-AIIR vs traditional patient room
- Medical Shelters (multi-beds)
- Portable LEV for aerosol-generating procedures
- Reverse Isolation ("Protective Isolation")
- Ambulance Ventilation
- Ambulance UVGI Decon
- Hospital Room Ventilation

37



Contract with Univ. of Cincinnati to evaluate and compare worker exposure potential in real AIIR vs regular patient room using CFD modeling

Multi-cot Shelter Applications

-Multi-bed version of expedient iso ventilated headboard sized for FMS cots
-Seeking to demonstrate concept with emergency response exercises
-Application in both "regular" and medical shelters
-Ease of construction can be enhanced with quick-connect ducting

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- Another view of multi-bed set-up
- Same cots as in SNS Stockpile
- Note new canopy design

•Now available in extruded aluminum construction

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Extruded Aluminum Design

- Commercially Available
- Lightweight/Sturdy
- Can become part of emergency response kit
- Adjustable height fits variety of cots or beds
- Easier to Clean/Decon

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Reverse Isolation Configuration

- **Recent testing inspired by Fukushima Nuclear plant incident**
- **HEPA airflow reversed to provide clean airflow over patient's torso**
- **Front curtain creates pos-pressure mini-environment**
- **Tested using aerosol spectrometers + modified version of respirator fit-testing method**

Results: > Iso Class 5 "Protection Factor" > 15,000

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Newest Development: Portable Isolation Hood (Crash-cart Concept)

- **Extruded Aluminum Frame**
- **Locking wheels**
- **Built-in fan/filter**
- **Capable of battery operation (90 minutes)**
- **Can operate off-grid via solar/wind energy**

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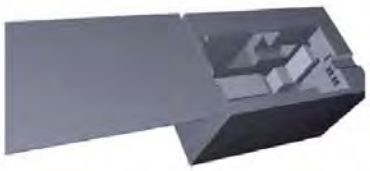
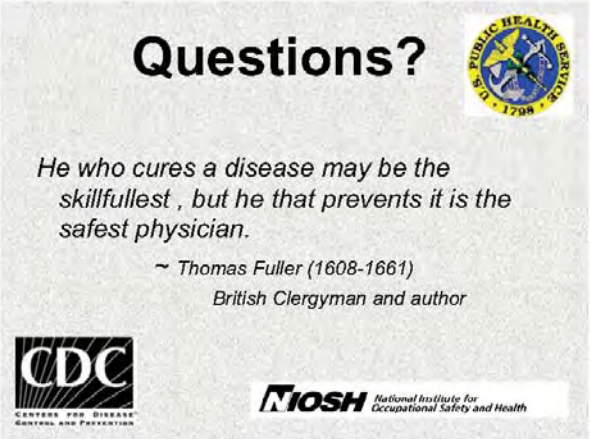
1. Engineering Controls For Emergency Medical Personnel
2. Tracer testing + CFD
3. Port. UVGI decon.?




Smoke Test in Ambulance Module
45


Questions?
He who cures a disease may be the skillfullest , but he that prevents it is the safest physician.
~ Thomas Fuller (1608-1661)
British Clergyman and author
CDC
NIOSH National Institute for Occupational Safety and Health

Update on Lab validation of new bioagent ID system: FilmArray

60 MDG

Maj Carlos Maldonado

In accordance with current (SGROCC #10000040) AFMS needs for advanced molecular diagnostic capabilities against infectious disease agents, the Clinical Investigation Facility (CIF) at Travis AFB, is participating in a multi-center, limited labora- tory validation (LLV) to assess both the utility and reliability of a new PCR platform in a variety of military settings. Idaho Technology's FilmArray system is a small (bread box-sized) PCR-based instrument capable of simultaneously detecting mul- tiple biological agents from a single clinical sample. This novel multiplex system also incorporates an initial sample purifica- tion step within the instrument eliminating the need for other equipment and a separate facility. The system's sample-to- answer turnaround time is approximately 1.25 hrs, which is a significant improvement over the 3-4 hours it takes for the currently fielded JBAIDS system. This study is sponsored by the AFMSA Research and Development Innovations (AFMSA/ SG9) office and Idaho Technology Inc. Learning Objectives:

Objective 1. List the current force health protection requirements of different MAJCOMs.

Objective 2. Discuss how the 43T clinical R&D is working to meet those force health protection requirements.

Objective 3. Discuss the advantages, limitations and mitigation strategies of molecular-based diagnostics.

Clinical Investigation Facility

Integrity - Service - Excellence

A Novel 'Spin' To an Old Technique…

Maj. Carlos J. Maldonado
Chief, Molecular Diagnostics
60 MDG/SGSE
Travis AFB, CA

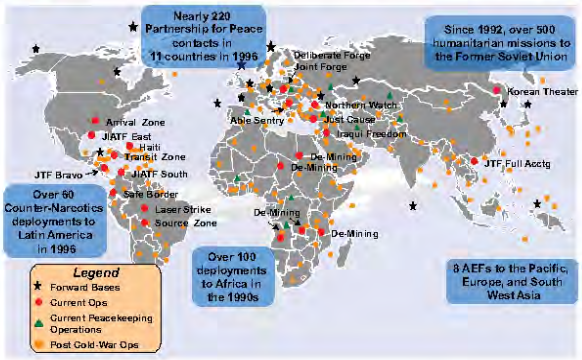
U.S. AIR FORCE

UNCLASSIFIED

Overview

- Current AFMS (infectious disease) Requirement
- Fielded Platform: M1M and JBAIDS
- What is Real-Time PCR?
- New System: FilmArray
- Multiplexing: Nested PCR
- New System's Capabilities, Specs, Pros and Cons
- What's next?

AEF: Operational Tempo

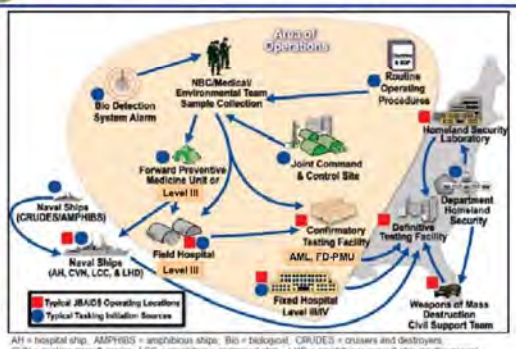


Current Medical Requirement

How do we monitor emerging/endemic infectious diseases and/or other biological agents?

MAJCOMS: ACC, AMC, AFSOC

Joint Medical Connectivity



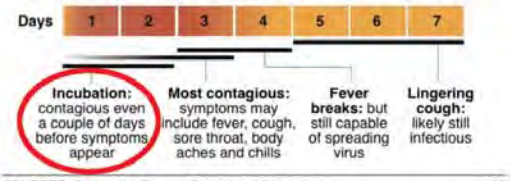
AH = hospital ship, AMPHIBS = amphibious ships, Bio = biological, CRUDES = cruisers and destroyers, CVN = nuclear aircraft carrier, LCC = amphibious command ship, LHD = amphibious assault ship (multipurpose), SOP = standard operating procedure

Pre-symptomatic 'shedding'

Contagious nature of virus may linger

How long people with swine flu stay infectious is not precisely known, but they may be able to spread it more than a week after symptoms first appear.

How doctors think swine flu progresses during contagious phase:



SOURCES: Centers for Disease Control and Prevention; interviews with infectious disease experts AP

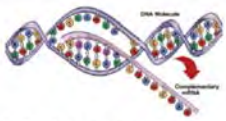
Biological Agents

(Operational Significance)

Levels of Identification

(Biological agents)

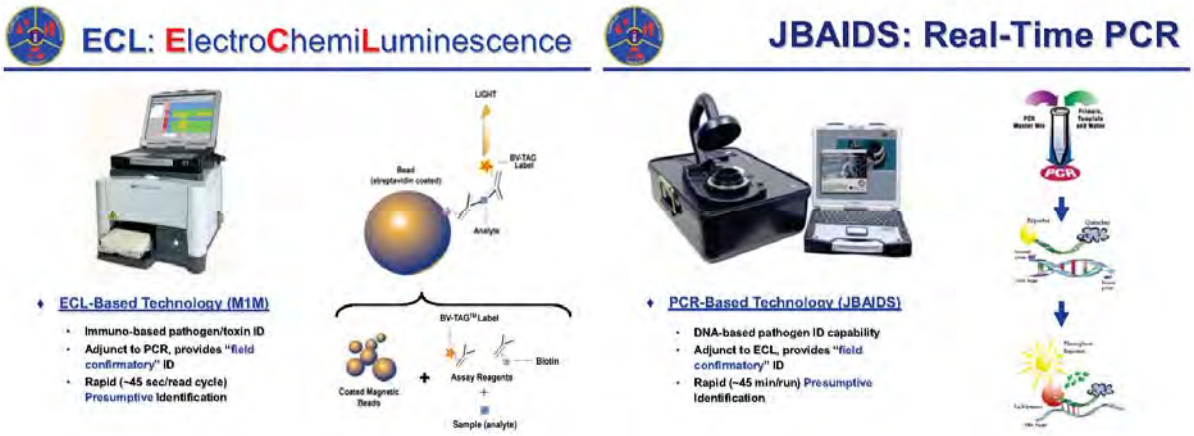
- **Nucleic Acid (DNA & RNA)**

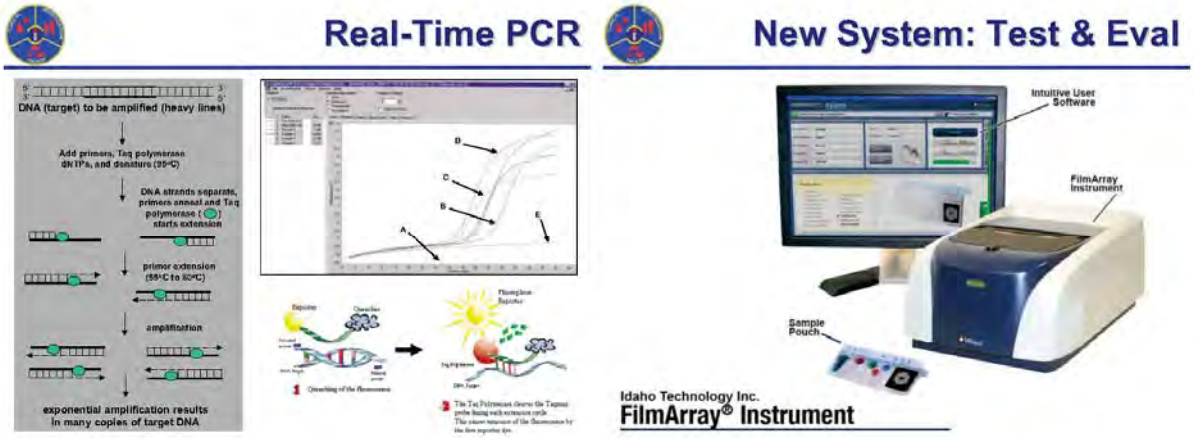


PCR-based assays

- **Protein (structural & secreted)**

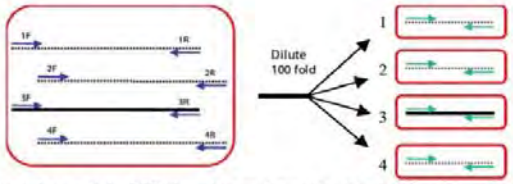
ECL-based assays


ECL: ElectroChemiLuminescence
ECL-Based Technology (M1M)
Immuno-based pathogen/toxin ID
Adjunct to PCR, provides "field confirmatory" ID
Rapid (~45 sec/read cycle) Presumptive Identification
LIGHT
BV-TAG Label
Bead (streptavidin coated)
Analyte
BV-TAG™ Label
Biotin
Assay Reagents
Coated Magnetic Beads
Sample (analyte)
JBAIDS: Real-Time PCR
PCR-Based Technology (JBAIDS)
DNA-based pathogen ID capability
Adjunct to ECL, provides "field confirmatory" ID
Rapid (~45 min/run) Presumptive Identification
PCR


Real-Time PCR
DNA (target) to be amplified (heavy lines)
Add primers, Taq polymerase dNTPs, and denature (95°C)
DNA strands separate, primers anneal and Taq polymerase ( ) starts extension
primer extension (55°C to 60°C)
amplification
exponential amplification results in many copies of target DNA
A
B
C
D
E
New System: Test & Eval
Intuitive User Software
FilmArray Instrument
Sample Pouch
Idaho Technology Inc.
FilmArray® Instrument

New System: 'Nested' PCR

Schematic of Nested Multiplex PCR

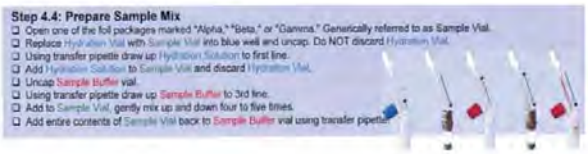


A large volume multiplex PCR (shown here as 4-plex on the left side of figure) is run for a limited number of cycles (20). The reaction is diluted and distributed to individual small real time PCR reactions that contain primers (green) nested inside the primers (blue) of the first PCR reaction. A template amplified in the first reaction (by the #3 primers) is further amplified in only one of the second reactions.

New System: 'Nested' PCR

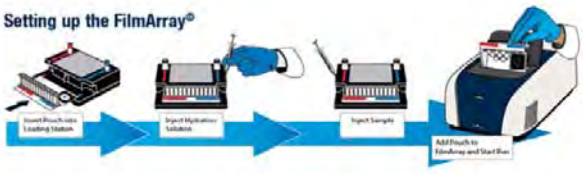
New System: Sample 'Prep'

- As tested by LLV instructions



Idaho Technology Inc.
FilmArray® Instrument

New System: Workflow



Idaho Technology Inc.
FilmArray® Instrument

New System: Results Report

FilmArray™
Respiratory Panel IVD

FilmArray
www.idahotech.com

Run Summary

Sample ID:	AdE4Hex-HPtoSS	**Run Date:**	10 Mar 2011 8:43 PM
Detected:	Adenovirus	**Controls:**	Passed
Equivocal:	None		

Result Summary

✓ Detected	Adenovirus
Not Detected	Coronavirus HKU1
Not Detected	Coronavirus NL63
Not Detected	Human Metapneumovirus
Not Detected	Human Rhinovirus/Enterovirus
Not Detected	Influenza A
Not Detected	Influenza B
Not Detected	Parainfluenza Virus 1
Not Detected	Parainfluenza Virus 2
Not Detected	Parainfluenza Virus 3
Not Detected	Parainfluenza Virus 4
Not Detected	Respiratory Syncytial Virus

Run Details

Pouch:	Respiratory Panel IVD v1.6	**Protocol:**	RPPv.7
Run Status:	Completed	**Operator:**	cli (cli)
Serial No.:	00071049	**Instrument:**	ITI FA "AFA21"
Lot No.:	100211		

New System: Specs

System Specifications

Sample Handling
- Sample Types: Nasopharyngeal Swab
- Sample Volume: 250 µL

Analytic Performance
- Sensitivity: Comparable to common singleplex molecular methods
- Specificity: Comparable to common singleplex molecular methods

Instrument Specifications
- Power Requirements: 90-264 VAC, 10 A
- Size: 25.4 x 29.3 x 16.5 cm (10 x 15.5 x 6.5 in.)

Weight: 9 kg (20 lb.)
- Performance Parameters
- Hands on time: Approx. 2 minutes
- Run turn-around time: 1 hour

Environmental Specification
- Operating: 15 °C to 30 °C at 20 to 80% humidity
- Storage: -30 °C to 65 °C

Desktop Software
- Windows-based instrument control and data analysis software
- Barcode reader for data input
- Automated qualitative analysis and reporting
- Separate advanced analysis software

New System: Targets

The FilmArray® Respiratory Panel

Simultaneous detection of 21 targets:

Viral: Adenovirus, Bocavirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus OC43, Coronavirus NL63, Enterovirus, Influenza A, Influenza A H1, Influenza A H1 2009, Influenza A H3, Influenza B, Metapneumovirus, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Parainfluenza 4, Respiratory Syncytial Virus, and Rhinovirus.

Bacterial: *Bordetella pertussis, Chlamydophila pneumoniae,* and *Mycoplasma pneumoniae.*

*FDA-cleared May 2011

News release

New System: Targets

FilmArray STI Panel

The FilmArray STI panel will provide a fast and compressive tool for testing sexually transmitted infections

- *N. gonorrhoeae*
- *C. trachomatis*
- *T. vaginalis*
- *M. genitalium*
- *U. parvum*
- *U. urealyticum*
- *T. pallidum HSV 1 & 2*
- *H. ducreyi*

FilmArray BioThreat Panel

The FilmArray BioThreat panel will provide a fast and compressive tool for testing biological threat pathogens

- *Bacillus anthracis*
- *Brucella species*
- *Burkholderia*
- *Clostridium botulinum*
- *Coxiella burnetii*
- *Ebola virus*
- *EEE virus*
- *Francisella tularensis*
- *Marburg virus*
- *Ricinus communis*
- *Rickettsia prowazekii*
- *Staphylococcus aureus*
- *Variola*
- *VEE virus*
- *WEE virus*
- *Yersinia pestis*

New System: Targets

New System: Expectations

- **Simple:** Automated protocol requires only two minutes of hands on time
- **Easy:** No precise measuring or pipetting required
- **Fast:** Turnaround time of one hour
- **Comprehensive:** 21 target respiratory panel

New System: Challenges

- Simultaneous testing of multiple samples (individuals)
- Complex clinical matrices: blood, sputum, stool, etc.
- Complex environmental matrices: soil, fatty food, pigments
- Real-world 'co-mingled' pathogen populations

JBAIDS Next Generation (FY17)

Key Performance Parameters

- Simultaneous ID of multiple toxins/pathogens
- In both clinical and environmental matrices
- Device and assays must be GMP compliant
- Required FDA approval (clinical diagnostic)
- Minimal logistics/personnel for operation
- Operate using minimal or no fluidics
- Reagentless systems are highly desirable
- Automated/integrated sample preparation
- Hand-held, ruggedized and of minimal weight
- Onboard software capable of device operation, output analysis, and information transfer


The Next Generation?
Medical tricorder
Is that a validated test?
Environmental Version?
Questions?
101 Bodin Circle
60th Medical Group
Clinical Investigation

Next-Generation Sequencing Technology for Disease Detection

711 HPW/USAFSAM-PHT

Dr. James Baldwin

Polymerase chain reaction (PCR) is a highly efficient method of pathogen detection; however, most PCR-based assays are unable to provide deeply multiplexed detections (25 or more). Furthermore, such tests need foreknowledge such as primers/probes in a PCR reaction. As a consequence, PCR tests are limited to a small number of potential known microbial targets and are not suitable for the detection of unexpected or newly emergent pathogens. We have demonstrated that methods such as degenerate PCR may be employed to detect larger selections of organisms, such as newly emergent threats, where exact primers are unknown. However, with increase in scope comes a greatly increased burden on the detection technology in the form of potentially numerous detections (deeply multiplexed) per sample. To meet the larger goal of detecting wide ranges of organisms in a manner suitable for clinical and environmental surveillance against biological threats, future assays will require enhanced equipment and software. The solution is next-generation sequencing technology. These devices can read many thousands to millions of parallel sequences in a single run (sample). Furthermore, they can produce exact sequences that are far more precise for identifying microorganisms than PCR alone. Recent advances could allow such platforms to approach the cost envelope of conventional PCR testing. Assays based on next-generation sequencing can provide the capability to detect rapidly emerging infections in deployed forces. A mature test in such a platform would offer a massive boost to the pathogen identification capabilities commonly available in the Air Force. Distribution Statement A: Approved for public release; distribution is unlimited. Case Number: 8ABW-2011-2230, 14 Apr 2011.

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Next-Generation Sequencing Technology for Disease Detection

AFMS Medical Research Symposium
August 3, 2011

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Emerging Infectious Disease

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Emerging infectious disease (EID) is a disease whose incidence has increased and threatens to increase in the near future.

- EID may account for at least 12% of all human pathogens.
- Some EIDs include diseases caused by a newly identified microorganism (e.g., SARS).
- Other EIDs can result from a change or evolution of an existing organism (e.g., influenza).
- Known infections that spread to a new area or population (e.g., West Nile virus) are also EIDs.

EID Is a Global Problem

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Upper Respiratory Viruses Cause Substantial Morbidity and Mortality

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- Over 52 strains of human adenovirus.
- Over 10 major strains of influenza.
- 5 strains of noteworthy coronavirus.
- Over 15 major classes of human pathogens in *Picornaviridae*.
- Most of these are not clinically relevant.
  - Infrequently seen.
  - Cause illness of limited severity.
- However, several strains are of the highest concern.
  - These viral serotypes can impact the health and readiness of military personnel.

Polymerase Chain Reaction Is a Rapid Way to Detect and Amplify DNA Sequences

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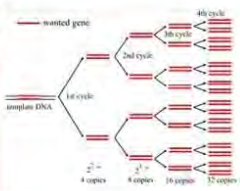

- Required specificity (or nonspecificity).
- Low complexity of sample handling.
- High throughput.

How Do We Get an Identification?

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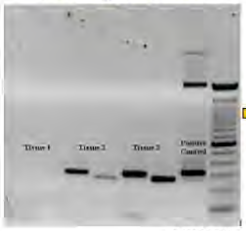
Getting from here…. To there…



PCR Gel Image, Roland Geddham

Oxoid Argus Product Literature

Limitations of PCR Alone

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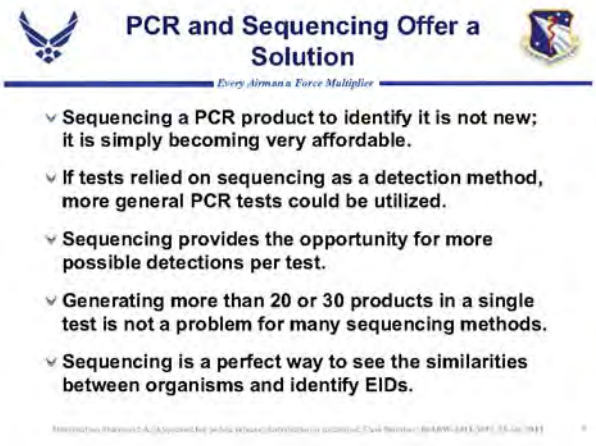
- PCR can easily be used to detect previously known infections.
- However, EID can be problematic. If the EID is a new strain, PCR will not always produce the expected results.
  - PCR will either erroneously detect this EID as the old infection, OR
  - PCR will fail to detect because the primers or probes don't match the EID.
- PCR alone simply does not give enough information to know if this has occurred.


PCR and Sequencing Offer a Solution
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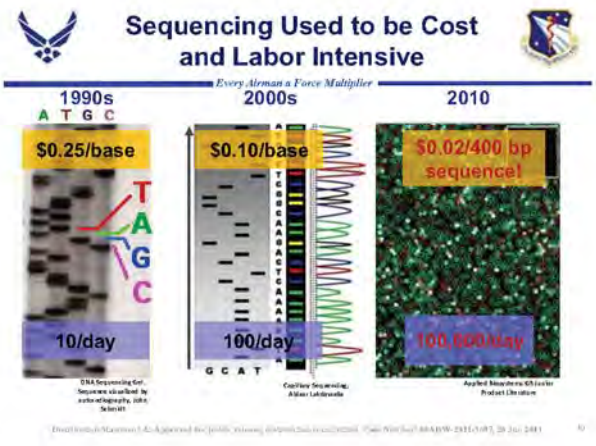
Sequencing a PCR product to identify it is not new; it is simply becoming very affordable.
If tests relied on sequencing as a detection method, more general PCR tests could be utilized.
Sequencing provides the opportunity for more possible detections per test.
Generating more than 20 or 30 products in a single test is not a problem for many sequencing methods.
Sequencing is a perfect way to see the similarities between organisms and identify EIDs.


Sequencing Used to be Cost and Labor Intensive
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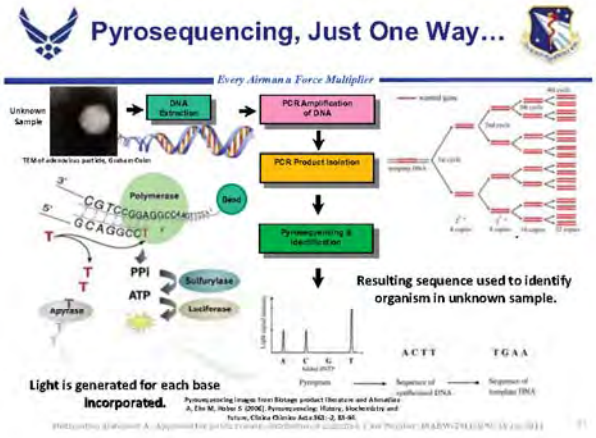
1990s
2000s
2010
A T G C
$0.25/base
$0.10/base
$0.02/400 bp sequence!
10/day
100/day
100,000/day


Pyrosequencing, Just One Way…
Every Airman a Force Multiplier
Unknown Sample
DNA Extraction
PCR Amplification of DNA
PCR Product Isolation
Polymerase
Bead
PPi
ATP
Sulfurylase
Luciferase
Apyrase
Resulting sequence used to identify organism in unknown sample.
ACTT
TGAA
Light is generated for each base incorporated.


Next-Generation Pyrosequencing Is Becoming Affordable
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There Are a Number of Platform Choices

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- Currently there are no less than four major vendors for this type of product. Most vendors have a range of devices to suit the needs of the customer. Prices range from $50,000-$400,000.
- Expect the cost to go down and the throughput to increase.

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Serotyping Detection Assays Suitable for EID

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- Bioinformatics analysis is used to determine the best genome sites for placing PCR test.
  - The analysis identified regions of similarity and divergence.
  - Primers (some degenerate) with the best thermodynamic properties in these regions were selected.
  - The divergent regions are sequenced.
  - The DNA sequence is usable as a barcode.

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Designing the Assays

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Proof of Concept: Upper Respiratory Virus Serotype Panel for the Pyrosequencer

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- To detect and serotype seven major classes of virus:
  - Coronavirus (including SARS)
    - 50 unique strains in GenBank
  - Human adenovirus (including 3,4,7,11,14,&21)
    - 52 unique strains in GenBank
  - Influenza A, B, & C virus (including pandemic N1H1)
    - 1358 unique strains in GenBank
  - Metapneumovirus
    - 13 unique strains in GenBank
  - Parainfluenza (including mumps and Sendai)
    - 25 unique strains in GenBank
  - *Picornaviridae* (coxsackievirus, echovirus, enterovirus, poliovirus, rhinovirus
    - 936 unique strains in GenBank
  - Respiratory syncytial virus
    - 12 unique strains in GenBank

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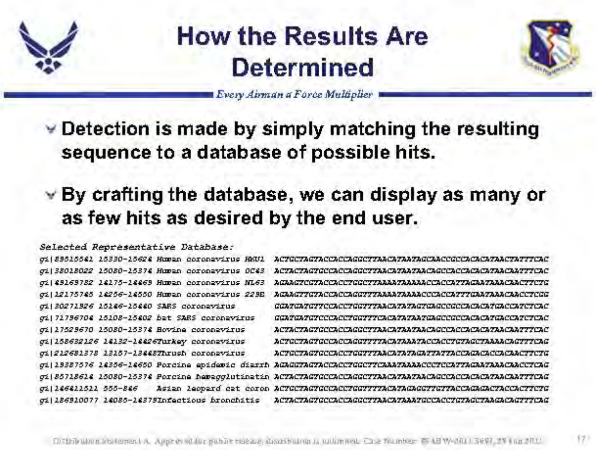
How the Results Are Determined
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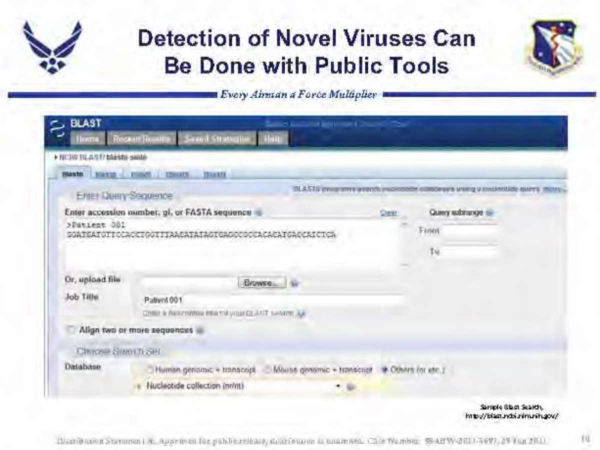
Detection is made by simply matching the resulting sequence to a database of possible hits.
By crafting the database, we can display as many or as few hits as desired by the end user.
Selected Representative Database:
gi|89515541 15330-15624 Human coronavirus HKU1 ACTGCTAGTACCACCAGGCTTAACATAATAGCAACCGCCACACATAACTATTTCAC
gi|38018022 15080-15374 Human coronavirus OC43 ACTACTAGTGCCACCAGGCTTAACATAATAACAGCCACCACACATAACAATTTCAC
gi|49169782 14175-14469 Human coronavirus NL63 AGAAGTCGTACCACCTGGCTTAAAATAAAAACCACCATTAGAATAAACAACTTCTG
gi|12175745 14256-14550 Human coronavirus 229E AGAAGTTGTACCACCAGGTTTAAAATAAAACCCACCATTTGAATAAACAACCTCGG
gi|30271926 15146-15440 SARS coronavirus GGATCATGTTCCACCTGGTTTAACATATAGTGAGCCGCCACACATGACCATCTCAC
gi|71796704 15108-15402 bat SARS coronavirus GGATGATGTCCCACCTGGTTTCACATATAATGAGCCGCCACACATGACCATCTCAC
gi|17529670 15080-15374 Bovine coronavirus ACTACTAGTGCCACCAGGCTTAACATAATAACAGCCACCACACATAACAATTTCAC
gi|158632126 14132-14426Turkey coronavirus ACTGCTAGTGCCACCAGGTTTTACATAAATACCACCTGTAGCTAAAACAGTTTCAG
gi|212681378 13157-13448Thrush coronavirus ACTGCTAGTGCCACCTGGTTTAACATATAGATTATTACCAGACACCACAACTTCTG
gi|19387576 14356-14650 Porcine epidemic diarrh AGAGGTAGTACCACCTGGCTTCAAATAAAACCCTCCATTAGAATAAACAACCTCAG
gi|85718614 15080-15374 Porcine hemagglutinatin ACTACTAGTGCCACCAGGCTTAACATAATAACAGCCACCACACATAACAATTTCAG
gi|146411511 555-846 Asian leopard cat coron ACTGCTAGTGCCACCTGGTTTTACATAGAGGTTGTTACCAGAGACTACCACTTCTG
gi|186910077 14085-14379Infectious bronchitis ACTACTAGTGCCACCAGGCTTAACATAAATGCCACCTGTAGCTAAGACAGTTTCAG


Detection of Novel Viruses Can Be Done with Public Tools
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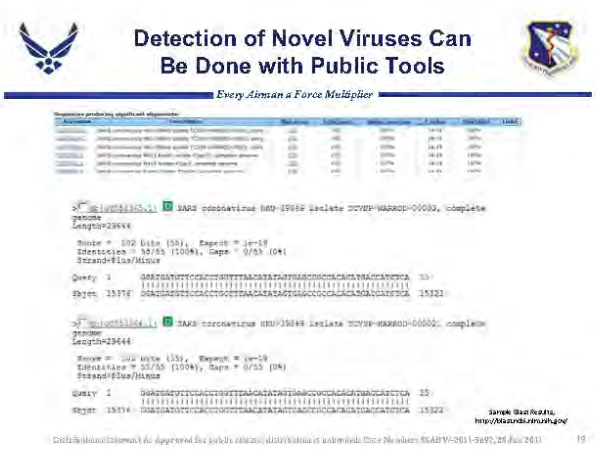
BLAST
Home Recent Results Saved Strategies Help
NCBI/ BLAST/ blastn suite
Enter Query Sequence
Enter accession number, gi, or FASTA sequence
>Patient 001
Query subrange
From
To
Or, upload file
Browse...
Job Title
Patient 001
Align two or more sequences
Choose Search Set
Database
Human genomic + transcript
Mouse genomic + transcript
Others (nr etc.)
Nucleotide collection (nr/nt)
Sample Blast Search,
http://blast.ncbi.nlm.nih.gov/


Detection of Novel Viruses Can Be Done with Public Tools
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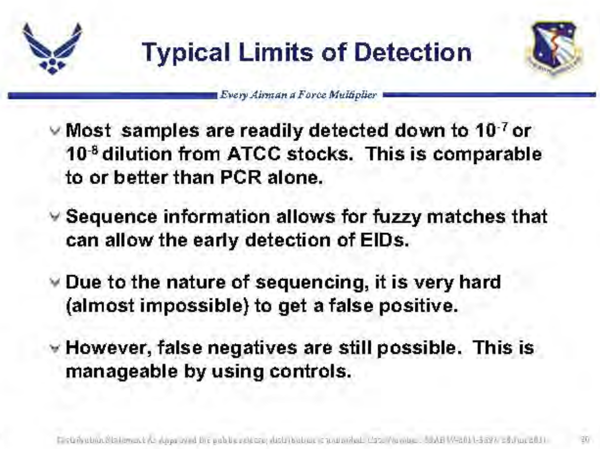
Sample Blast Results,
http://blast.ncbi.nlm.nih.gov/


Typical Limits of Detection
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Most samples are readily detected down to 10⁻⁷ or 10⁻⁸ dilution from ATCC stocks. This is comparable to or better than PCR alone.
Sequence information allows for fuzzy matches that can allow the early detection of EIDs.
Due to the nature of sequencing, it is very hard (almost impossible) to get a false positive.
However, false negatives are still possible. This is manageable by using controls.

Proof of Concept Assays Show Merit

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- Degenerate PCR followed by next-generation sequencing methodology offers several unique directions for future assays.
  - Allows deep multiplexing.
  - Is amenable to several different instruments.
  - Can readily detect large subsets of similar organisms.
  - Can individually identify members of these groups.

Future Directions

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Deep multiplexing with next-generation sequencing offers the same diversity of sample types as conventional PCR.

Sequencing assays are more tolerant of multiple detections.

Problems to Overcome

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- Assay complexity – Sequencing is one more step than PCR.
- Cost – This will be reduced, but right now it is too expensive for routine testing.
- Bioinformatics can readily serotype today. However, more tools will be needed to easily identify new EIDs.
- Customer acceptance of slightly more complex results.
- FDA clearance.

FHT Mission

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Provide continual and rapid evaluation, validation, and transition assistance of new off-the-shelf technologies and identify emerging technologies ("technology discovery") to fill critical gaps in force protection, rapid diagnostics, epidemiology, and preventive medicine, including CBRNE identification, to meet Air Force global mission requirements.

Acknowledgments

Every Airman a Force Multiplier

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- Ms. Sandy Kawano

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